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1. Document ID: AU 9950311 A, DE 19900503 A1, WO 200040263 A1

L2: Entry 1 of 2

File: DWPI

Jul 24, 2000

DERWENT-ACC-NO: 2000-466995

DERWENT-WEEK: 200052

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TITLE: Use of composition containing anti-Fas antibody, for treating e.g. toxic epidermal necrolysis or hepatitis, inhibits interaction between Fas receptor and ligand

INVENTOR: FRENCH, L E; TSCHOPP, J; VIARD, I; FRENCH, E L

PRIORITY-DATA: 1999DE-1000503 (January 8, 1999)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9950311 A	July 24, 2000	N/A	000	A61K039/395
DE 19900503 A1	July 13, 2000	N/A	019	A61K039/395
WO 200040263 A1	July 13, 2000	G	000	A61K039/395

INT-CL (IPC): A61K 39/395; C07K 16/06; C07K 19/00; G01N 33/68

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KVMC	Drawl Desc	Image
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- ☐
2. Document ID: AU 9887996 A, DE 19725847 A1, WO 9857992 A2

L2: Entry 2 of 2

File: DWPI

Jan 4, 1999

DERWENT-ACC-NO: 1999-061508  
DERWENT-WEEK: 199921  
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TITLE: New antibody to FLIP protein - used to suppress inhibition of apoptotic signal transduction by FLIP proteins, to detect FLIP proteins and to screen for substances that activate FLIP expression

INVENTOR: BODMER, J; BURNS, K ; FRENCH, E ; HAHNE, M ; HOFFMANN, K ; IRMLER, M ; RIMOLDI, D ; SCHNEIDER, P ; SCHROETER, M ; STEINER, V ; THOME, M ; TSCHOPP, J; FRENCH, E L

PRIORITY-DATA: 1997DE-1025847 (June 18, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9887996 A	January 4, 1999	N/A	000	C07K016/00
DE 19725847 A1	December 24, 1998	N/A	013	C07K016/00
WO 9857992 A2	December 23, 1998	G	000	C07K016/00

INT-CL (IPC): C07K 16/00; C12N 1/00; C12N 5/10; C12N 15/11; C12N 15/63

Full	Title	Citation	Front	Review	Classification	Date	Reference
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(fas ligand) near100 (gvdh or graft versus host)	1658

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versus host)

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## Search History

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USPT,PGPB,JPAB,EPAB,DWPI	(fas ligand) near 200 (gvdh or graft versus host)	365002	<u>L3</u>
DWPI,EPAB,JPAB,PGPB,USPT	fas and l1	2	<u>L2</u>
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AND CA  
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=> s l2 and (graft versus host or gvh?)

L3 88 L2 AND (GRAFT VERSUS HOST OR GVHD)

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=> d14 1 37 bib ab

L4 ANSWER 1 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)

AN 2001 312281 SCISEARCH

GA The Genome Article (R) Number 421PM

TI Transgenic mice ubiquitously expressing human Fas ligand

develop a slight form of graft-versus host

-like disease

AU Ma Y H, Fei J, Hu J H, Zhou X G, Xia G H, Guo L H (Reprint)

CS Chinese Acad Sci, Shanghai Inst Biol Sci, Inst Biochem & Cell Biol,

Shanghai 200031, Peoples R China (Reprint)

CVA Peoples R China

SO ACTA PHARMACOLOGICA SINICA (APR 2001) Vol. 22, No. 4, pp 311-

319

Publisher: ACTA PHARMACOLOGICA SINICA, 294 TAIYUAN ROAD,

SHANGHAI 200031,

PEOPLES R CHINA

ISSN 0253-9756

DT Article, Journal

LA English

REC Reference Count 43

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB AIM To construct transgenic mice bearing human Fas

ligand (Fas/CD95L) cDNA, and further explore the physiological

effects of ubiquitous expression of Fas on such animals. METHODS

Transgenic mice were produced by pronuclear microinjection method

Integration and transmission of transgene were identified by nest-PCR and

Southern-blot analysis. Level of Fas mRNA was evaluated by

semi-quantitative RT-PCR analysis. Fast protein was detected by

immunofluorescence analysis. Morphological alterations in tissues were

analyzed by histological examination. The percentage of alpha beta T cells

in the spleen was determined by flow cytometry analysis. RESULTS Two

independent founder mice bearing human Fas cDNA under the control of

CMV promoter were generated healthily. Human Fas was moderately expressed

in the majority of tissues examined in F1 heterozygotic mice. Although

developing normally, adult transgenic mice exhibited a slight form of

in recipients receiving nonmyeloablative conditioning. As compared with donor lymphocytes given early post-BMT, DLs are associated with a reduced risk of graft-versus-host disease (GVHD). The mechanism(s) responsible for such resistance have remained incompletely defined. We now have observed that host T cells present 3 wk after lethal total body irradiation, at the time of DL, contribute to DL-GVHD resistance. The infusion of donor splenocytes on day 0, a time when host bone marrow (BM)-derived T cells are absent, results in greater expansion than later post-BMT when host and donor BM-derived T cells coexist. Selective depletion of host T cells with anti-Thy1 allelic mAb increased the GVHD risk of DL, indicating that a Thy1+ host T cell regulated DL-GVHD lethality. The conditions by which host T cells are required for optimal DL resistance were determined. Recipients unable to express CD28 or 4-1BB were as susceptible to DL-GVHD as anti-Thy1 allelic mAb-treated recipients, indicating that CD28 and 4-1BB are critical to DL-GVHD resistance. Recipients deficient in both perforin and Fas ligand but not individually were highly susceptible to DL-GVHD. Recipients that cannot produce IFN- $\gamma$  were more susceptible to DL-GVHD, whereas those deficient in IL-12 or p55 TNFRI were not. Collectively, these data indicate that host T cells, which are capable of generating antitumor CTL effector cells, are responsible for the impaired ability of DL to induce GVHD. These same mechanisms may limit the efficacy of DL in cancer therapy under some conditions.

L4 ANSWER 3 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)

AN 2000 280490 SCISEARCH

GA The Genome Article (R) Number 288GC

TI Blockade of CD134 (OX40)/CD134L interaction ameliorates lethal acute

graft-versus-host disease in a murine model of

allogeneic bone marrow transplantation

AU Tsukada N, Akiba H, Kobata T (Reprint), Azawa Y, Yagita H, Okumura K

CS DOKKO UNIV, SCH MED, INST MED SCI, DIV IMMUNOL, 860

KITAKOAWASHI, MIBU,

TOCHIGI 3210293, JAPAN (Reprint), DOKKO UNIV, SCH MED, INST MED

SCI, DIV

IMMUNOL, MIBU, TOCHIGI 3210293, JAPAN, JUNTENDO UNIV, SCH MED,

DEPT

IMMUNOL, TOKYO 113, JAPAN, NIKGATA UNIV, SCH MED, DEPT

INTERNAL MED 1,

NIGATA, JAPAN, JST, CREST, TOKYO, JAPAN

CVA JAPAN

SO BLOOD (1 APR 2000) Vol. 95, No. 7, pp 2434-2439

Publisher: AMER SOC HEMATOLOGY, 1200 19TH ST, NW, STE 300,

WASHINGTON, DC

20036-2422

ISSN 0006-4871

DT Article, Journal

LA English

REC Reference Count 47

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Expression of CD134 (OX40) on activated CD4(+) T cells has been

observed in acute graft-versus-host disease

(GVHD) after human and rat allogeneic bone marrow transplantation (BMT). We investigated the role of interaction between CD134 and CD134 ligand (CD134L) in a murine model of acute GVHD by using a newly established monoclonal antibody (mAb) against murine CD134L. Acute GVHD was induced by transfer of bone marrow cells and spleen cells into lethally irradiated recipients in a parent (C57BL/6) to first filial generation (C57BL/6 crossed with DBA/2) BMT. Administration of anti-CD134L mAb significantly reduced the lethality of acute GVHD and other manifestations of the disease, such as loss of body weight, hunched posture, diarrhea, and patchy alopecia. The survival rate 80 days after BMT in mice treated with the mAb was about 70%, whereas all mice treated with control antibodies died within 43 days. Histologic examinations revealed that inflammatory changes in target organs such as the liver, gut, and skin were also ameliorated in mice treated with the mAb compared with control mice. An in vitro assay of T-cell proliferation showed a marked hyporesponsiveness to host antigen in samples from mice treated with anti-CD134L mAb. In addition, low levels of interferon gamma and transiently elevated levels of interleukin 4 and IL6 in serum samples were found in mice treated with anti-CD134L mAb. These results suggest that CD134-CD134L interactions have

an important role in the pathogenesis of acute GVHD. (Blood 2000 95:2434-2439) (C) 2000 by The American Society of Hematology

L4 ANSWER 4 OF 37 MEDLINE

DUPLICATE 2

AN 2000386494 MEDLINE

DN 20055481 PubMed ID 10898516

T1 Double mutant MRL-*lpr/lpr*-gld/gld cells fail to trigger *lpr* graft

-versus-host disease in syngeneic wild-type recipient

mouse, but can induce wild-type B cells to make autoantibody

AU Zhu B, Beaudette B C, Rifkin I R, Marshak-Roshien A

CS Department of Microbiology, Boston University School of Medicine, MA

02118, USA

NC 035230 (NIAHIS)

DK02597 (NIDDK)

SO EUROPEAN JOURNAL OF IMMUNOLOGY (2000 Jun) 30 (6): 1778-84

Journal code EN5: 1273201 ISSN: 0014-2980

CY GERMANY Germany, Federal Republic of

DT Journal, Article, (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 2000008

ED Entered STN 20000818

Last Updated on STN 20000818

Entered Medicine 20000809

Lethally irradiated mice reconstituted with histocompatible stem cells

from Fas-deficient MRL/lpr mice develop a wasting syndrome reminiscent of

chronic graft-versus-host disease. However,

reconstitution with double Fas-/Fas ligand

(FasL)-deficient stem cells does not result in wasting disease,

demonstrating that FasL expression is an important component of the

effector mechanisms leading to this syndrome in the absence of wasting

disease double-deficient T cells can induce wild-type B cells to make

autoantibodies. These data indicate that autoantibody production is

regulated by FasL-expressing T cells, and that Fas-sufficient wild-type B

cells differ from Fas-deficient lpr cells only with regard to their

sensitivity to FasL

L4 ANSWER 5 OF 37 BIOSIS COPYRIGHT 2001 BIOSIS

AN 2001 15398 BIOSIS

DN PREV200100015298

T1 Fas-mediated cell death in toxic epidermal necrolysis and graft-

versus-host disease: Potential for therapeutic

inhibition

AU French L E, Tschopp J (1)

CS (1) Institute of Biochemistry, Lausanne University, CH-1066, Epalinges

Jung Tschopp (2) b unit ch Switzerland

SO Schweizerische Medizinische Wochenschrift, (4 November, 2000) Vol

130

No. 44, pp. 1656-1661, print

ISSN 0036-7672

DT Article

LA English

Death receptors are a growing family of transmembrane proteins which can

detect the presence of specific extracellular death signals and rapidly

trigger cellular destruction by apoptosis. The best studied to date is Fas

(CD95). Expression and signaling for key physiological functions in a

tightly regulated process essential for the maintenance of immune homeostasis

variety of organs, including the maintenance of immune homeostasis

Recently, strong evidence has shown that dysregulation of Fas expression

and/or signaling contributes to the pathogenesis of toxic epidermal

necrolysis and acute graft-versus-host

disease. With these new developments, strategies for modulating the

function of Fas signaling have emerged and opened up novel therapeutic

possibilities. Specific blockade of Fas, for example with intravenous

immunoglobulin preparations containing specific anti-Fas

antibodies, has shown great promise in the treatment of toxic

epidermal necrolysis and may also be useful in the treatment of acute

graft-versus-host disease. Further

developments in this field may have important clinical implications for

the treatment of such diseases

L4 ANSWER 6 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

AN 2001018875 EMBASE

T1 Deengagement of apoptosis-related lymphocyte homeostasis in systemic

sclerosis

AU Stummvoll G H, Aringer M, Smolen J S, Kotler M, Krenel H P, Stener

C W, Bohle B, Knobler R, Gminger W B

CS W B Gminger, Department of Rheumatology, Internal Medicine III,

University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

SO Rheumatology, (2000) 39(12):1341-1350

Reis 57

ISSN 1462-0324 CODEN RUMAFK

CY United Kingdom

DT Journal, Article

FS 005 General Pathology and Pathological Anatomy

026 Immunology, Serology and Transplantation

031 Arthritis and Rheumatism

037 Drug Literature Index

LA English

SL English

AB Objectives: Both increased and decreased apoptosis may be involved in

generating autoimmunity. This study addressed the question of whether

apoptosis and apoptosis-regulating proteins are altered in systemic

sclerosis (SSc). Patients and methods: Peripheral lymphocytes of 39 SSc

patients and 47 healthy control persons were studied for apoptosis. Bcl-2

and Bax levels, expression of Fas (CD95) and activation markers (CD25,

HLA-DR) as determined by flow cytometry. Serum Fas and Fas

ligand were measured by ELISA. Results: SSc lymphocytes (mainly

CD4(+)) expressed increased amounts of Bcl-2, while Bax was not elevated

Apoptosis rates of SSc lymphocytes were increased in unsupplemented

medium, but returned to normal in the presence of autologous plasma. SSc

patients had increased percentages of activated and CD95(+) lymphocytes

and elevated soluble Fas and soluble FasL levels in serum. Activating

anti-CD95 antibodies further increased the apoptosis rate.

Conclusions: Increased in vitro apoptosis, elevated lymphocyte Bcl-2

content and the increased number of Fas-positive T cells are not specific

for peripheral blood from SSc patients, but indicate deregulation of

lymphocyte homeostasis in this disease

L4 ANSWER 7 OF 37 MEDLINE

AN 2001102227 MEDLINE

DN 200409065 PubMed ID 10953977

T1 A metalloproteinase inhibitor prevents acute graft

versus-host disease while preserving the

graft-versus-leukaemia effect of allogeneic bone marrow transplantation

AU Hattori K, Hirano T, Oshimi K, Yagita H, Okumura K

CS Department of Internal Medicine, Juntendo University School of Medicine,

Tokyo, Japan

SO LEUKEMIA AND LYMPHOMA, (2000 Aug) 38 (5-6):553-61

Journal code BNC ISSN: 1042-8194

CY Switzerland

DT Journal, Article, (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200101

ED Entered STN 20010322

Last Updated on STN 20010322

Entered PubMed 20010123

Entered Medicine 20010126

AB Tumor necrosis factor (TNF) and Fas ligand (FasL) have

been implicated in the pathogenesis of graft-versus-

host disease (GVHD). Several recent studies have shown

that some metalloproteinase mediates TNF-alpha and FasL processing. We

examined the ameliorating effect of a hydroxamic acid-based

metalloproteinase inhibitor (KB-R7785) that inhibits TNF-alpha and FasL

release in a lethal acuteGVHD model in mice. The ameliorating effect of

KB-R7785 was superior to that of anti-TNF-alpha antibody. We

also examined the effect of KB-R7785, which we previously demonstrated a

potent ameliorating effect on acute GVHD, on

graft-versus-leukemia (GVL) effect of allogeneic bone marrow

transplantation (BMT). Administration of KB-R7785 without bone marrow

transplantation (BMT) significantly prolonged the survival of

10E-producing B53 hybridoma cell-inoculated (C57BL/6 x BALB/c) F1 (CBF1)

mouse by inhibiting the initiation of B53 cells into the liver and

spleen. Transplantation of B6 BMS without KB-R7785 resulted in the death

of most recipients due to acute GVHD while efficiently

eliminating B53 cells. Administration of KB-R7785 along with B6 BMS

resulted in 50% survival of B53-inoculated CBF-1 mice over 50 days without

histological manifestations of acute GVHD or residual B53 cells.

These results suggest that KB-R7785 could be a potent therapeutic agent

for GVHD, and indicate the beneficial effects of KB-R7785 that

inhibit tumor infiltration and prevent acute GVHD while

preserving the GVL effect of allogeneic BMT

L4 ANSWER 8 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

AN 2000418994 EMBASE

T1 Anti-third party CD8+ CTLs as potent veto cells: Coexpression of CD8 and

FasL is a prerequisite

AU Reich-Zenger S, Zhao Y, Krautinger R, Bachar-Lusby E, Reissner Y

CS Y Reissner, Department of Immunology, Weizmann Institute of Science,

Rehovot 76100, Israel, yair.reissner@weizmann.ac.il

SO Immunology, (2000) 13(4):507-515

Reis 58

ISSN 1074-7613 CODEN IJNIEH

CY United States

DT Journal, Article

FS 026 Immunology, Serology and Transplantation

LA English

SL English

AB Several bone marrow cells and lymphocyte subpopulations, known as 'veto

cells', were shown to induce transplantation tolerance across major

histocompatibility antigens. Recently, it has been suggested that

anti-third party CTLs depleted of alloreactivity are endowed with marked

veto activity and therefore might potentially facilitate bone marrow

allografting without graft-versus-host

disease (GVHD). The veto mechanism is still obscure. While early

studies emphasized the role of CD8-mediated apoptosis, more recent

evidence indicates a role for Fas-FasL. In the present study we show, by

using blocking anti-CD8 antibody, by generating CTLs from FasL

or perforin mutated mice, and by gene transfer of FasL, that the veto

activity of anti-third party CD8+ CTLs is dependent upon the simultaneous

expression of both CD8 and FasL

L4 ANSWER 9 OF 37 MEDLINE

AN 200035783 MEDLINE

DN 20356783 PubMed ID 10901605

T1 T-cell co-signaling molecules in graft-versus-

host disease

AU Tanaka J, Asaka M, Imamura M

CS Department of Haematology and Oncology, Hokkaido University School of

Medicine, Sapporo, Japan

SO ANNALS OF HEMATOLOGY, (2000 Jun) 79 (6):283-90. Ref: 80

Journal code A2P: 9107334 ISSN: 0959-5555

CY GERMANY Germany, Federal Republic of

DT Journal, Article, (JOURNAL ARTICLE)

General Review (REVIEW)

(REVIEW, TUTORIAL)

LA English

FS Priority Journals

EM 200007

ED Entered STN 20000811

Last Updated on STN 20000811

Entered Medicine 20000728

AB Allogeneic stem cell transplantation (allo SCT) is now frequently

performed for the treatment of haematological malignancies and aplastic

anaemia. However, graft-versus-host disease

(GVHD) is still the major complication after allo SCT, producing

immune deficiency, infection, organ damage and, occasionally, patient

death. The antigen-specific signal mediated by the T-cell receptor (TCR)

is essential for activation of T-cells; however, additional co-stimulatory

signals are required for complete T-cell activation. Therefore, blocking

strategies of co-stimulatory signals have been evaluated as targets of

therapeutic intervention for GVHD after allo SCT. In a mouse

bone-marrow transplantation (BMT) model, the administration of CTLA4-Ig,

which blocks the interaction of CD28 on T-cells and B7 molecules on

antigen-presenting cells (APCs), can prolong survival of allo BMT

recipients, although this effect was not complete. In addition, the

anti-CD40L (CD154) monoclonal antibody (mAb), which

can interfere with the interaction of CD154 on T-cells and CD40 on APCs,

can induce long term graft survival in the murine model. Combined

administration of CTLA4-Ig and anti-CD40L mAb can prevent allograft

rejection in primates. Therefore, it seems the most powerful method to

prevent the alloimmune response in vivo. The Fas/Fas

ligand pathway is also involved in pathogenesis of GVHD

Anti-FasL mAb can reduce the mortality of GVHD and improve

intestinal lesions. Recently, it was reported that donor bone marrow

treated ex vivo using CTLA4-Ig reconstituted haematopoiesis in vivo with a

relatively low risk of GVHD in human allo BMT. Therefore, selective blocking strategies for T cell co-signaling might be useful for the prevention of GVHD in human allo SCT.

L4 ANSWER 10 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI B.V. DUPLICATE 5  
AN 2000391287 EMBASE

TI Effect of a matrix metalloproteinase inhibitor on host resistance against Listeria monocytogenes infection  
AU Yamada K, Yoshino K, Sekikawa K, Madarame H, Yagita H, Nakane A, CS A Nakane, Department of Bacteriology, Hirotsuki University Sch of Medicine, Zaitu-cho 5, Hirotsuki, Aomori 036-8562, Japan  
a27k03n06cc hirotsuki-u.ac.jp  
SO FEBS Immunology and Medical Microbiology (4 Nov 2000) 29(3):187-194  
Refs 40 CODEN FIMIEV  
ISSN 0928-8244 CODEN FIMIEV  
PUI 5 0928-8244(00)00204-2

CY Netherlands  
DT Journal Article  
FS 004 Microbiology  
037 Drug Literature Index  
LA English  
SL English

TI Hydroxy acid-based matrix metalloproteinase (MMP) inhibitors have been shown to inhibit tumor infiltration and growth, endotoxin shock, and acute graft-versus-host disease. Blockade of the

release of soluble tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and CD95 ligand (CD95L, FasL) from cell-associated forms is reportedly involved in the mechanism of the drug effect. We investigated the effect of a MMP inhibitor, KB-R7785, on host resistance against *Listeria monocytogenes* infection, in which TNF- $\alpha$  is essentially required for the defense. In mice, the administration of KB-R7785 exacerbated sepsis, while the drug prevented lethal shock induced by lipopolysaccharide and D-galactosamine. KB-R7785 inhibited soluble TNF- $\alpha$  production in spleen cell cultures stimulated by heat-killed *L. monocytogenes* and the drug treatment reduced serum TNF- $\alpha$  levels in infected mice, whereas the compound was ineffective on the modulation of interferon- $\gamma$  and interleukin-10 production. The effect of KB-R7785 was considered to be dependent on TNF- $\alpha$  because the drug failed to affect *L.*

monocytogenes infection in anti-TNF- $\alpha$  monoclonal antibody-treated mice and TNF- $\alpha$  knockout mice. Anti-CD95L monoclonal antibody was also ineffective on the infection. These results suggest that induction of infectious diseases, to which TNF- $\alpha$  is critical in host resistance, should be considered in MMP inhibitor-treated hosts. (C) 2000 Federation of European Microbiological Societies

L4 ANSWER 11 OF 37 MEDLINE DUPLICATE 6  
AN 2000429103 MEDLINE  
DN 20409099 PubMed ID 10961228

TI Death receptors in cutaneous biology and disease  
AU Wehrli P, Vardi I, Buliam R, Tschopp J, French L E  
CS Department of Dermatology, Geneva University Medical School, Geneva, Switzerland  
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY (2000 Aug) 115 (2):141-8 Ref 87

CY United States  
DT Journal Article, (JOURNAL ARTICLE)  
General Review, (REVIEW)  
(REVIEW TUTORIAL)

LA English  
FS Priority Journals  
EM 200009  
ED Entered STN 20000922  
Last Updated on STN 20000922

AB Death receptors are a growing family of transmembrane proteins that can detect the presence of specific extracellular death signals and rapidly trigger cellular destruction by apoptosis. Expression and signaling by death receptors and their respective ligands is a tightly regulated process essential for key physiologic functions in a variety of organs, including the skin. Several death receptors and ligands, Fas and Fas ligand being the most important to date, are expressed in the skin and have proven to be essential in contributing to its functional integrity. Recent evidence has shown that Fas-induced keratinocyte apoptosis in response to ultraviolet light prevents the

accumulation of pro-carcinogenic p53 mutations by deleting ultraviolet-mutated keratinocytes. Further, more there is strong evidence that dysregulation of Fas expression and/or signaling contributes to the pathogenesis of toxic epidermal necrolysis, acute cutaneous graft-versus-host disease, contact hypersensitivity and melanoma metastasis. With these new developments, strategies for modulating the function of death receptor signaling pathways have emerged, and provided novel therapeutic possibilities. Specific blockade of Fas, for example with intravenous immunoglobulin preparations that contain specific anti-Fas antibodies, has shown great promise in the treatment of toxic epidermal necrolysis and may also be useful in the treatment of acute graft-versus-host disease.

Likewise, induction of death signaling by ultraviolet light can lead to hapten-specific tolerance, and gene transfer of Fas ligand to dendritic cells can be used to induce antigen specific tolerance by deleting antigen-specific T cells. Further developments in this field may have important clinical implications in cutaneous disease.

L4 ANSWER 12 OF 37 MEDLINE DUPLICATE 7  
AN 2000118144 MEDLINE  
DN 20118144 PubMed ID 10651946

TI Graft-versus-host disease-associated donor cell engraftment in an F1 hybrid model is dependent upon the Fas pathway.  
AU Iwasaki T, Hamano T, Sasaki K, Kuwano T, Katsuda Y, Takemoto Y, Ogata A.

CS The Second Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.  
SO IMMUNOLOGY (2000 Jan) 98 (1):94-100.  
Journal code GH7 0374672 ISSN 0019-2805

CY ENGLAND United Kingdom  
DT Journal Article, (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 200002  
ED Entered STN 20000218  
Last Updated on STN 20000218

AB The graft-versus-host disease (GVHD) generated in BDF1 mice by the injection of spleen cells from the C57BL/6 parental strain induces a direct cell-mediated attack on host lymphohaematopoietic populations, resulting in the reconstitution of the host with donor cells. We examined Fas-Fas ligand (FasL) interactions in donor and host haematopoietic cells over a prolonged period of parental-induced GVHD. Fas expression on bone marrow cells of both donor and host origin increased at 2 weeks. Host cell incubation with anti-Fas antibody induced apoptosis, and the number of haematopoietic progenitor cells decreased. Fas-induced apoptosis by the repopulating donor cells, however, did not increase until 12 weeks, when more than 90% of the cells were donor cells. The expression of various cytokines, such as interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and FasL gene expression in the bone marrow increased concomitantly. To examine directly whether FasL has a major role in the development of donor cell engraftment, FasL-deficient (gld) mice were used as donors. Injection of B6.gld spleen cells induced significantly less host lymphohaematopoietic depletion, resulting in a failure of donor cell engraftment. Furthermore, injection of IFN- $\gamma$  and FasL gene knockout (gko) B6 spleen cells failed to augment Fas and FasL expression in recipient mice, resulting in a failure of donor cell engraftment. This suggests that the induction of apoptosis by Fas-FasL interactions in host cells may contribute to a reconstitution of the host with donor cells and that donor-derived IFN- $\gamma$  plays a significant role for Fas-FasL interactions in host cells during parental-induced GVHD.

L4 ANSWER 13 OF 37 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 8  
AN 2000 454060 BIOSIS  
DN PREV200000454060

TI Role of perforin in controlling B-cell hyperactivity and humoral autoimmunity.  
AU Shustov, Andreev, Luzina, Ima, Nguyen, Phuong, Pasadimitou, John C., Handwerker, Barry, Elkon, Keith B., Via, Charles S. (1)

CS (1) Division of Rheumatology and Clinical Immunology, University of Maryland School of Medicine, 10 S. Pine Street, MSF1B-34, Baltimore, MD, 21201 USA

SO Journal of Clinical Investigation. (September, 2000) Vol 106 No 6, pp R39-R47 print  
ISSN 0021-9738

DT Article  
LA English  
SL English

AB To determine the role of perforin-mediated cytotoxic T lymphocyte (CTL) effector function in immune regulation, we studied a well-characterized mouse model of graft-versus-host disease (GVHD).

GVHD induction of acute GVHD using perforin-deficient donor T cells (pfpwawv1) initially resulted in features of acute GVHD, e.g., engraftment of both donor CD4+ and CD8+ T cells, upregulation of Fas and FasL, production of antihistone CTL, and secretion of both Th1 and Th2 cytokines. Despite fully functional FasL activity, pfpwawv1 donor cells failed to totally eliminate host B cells, and, by 4 weeks of disease, cytokine production in pfpwawv1 mice had polarized to a Th2 response. Pfpwawv1 mice eventually developed features of chronic GVHD, such as increased numbers of B cells, persistence of donor CD4+ T cells, autoantibody production, and lupuslike renal disease. We conclude that, in the setting of B- and T-cell activation, perforin plays an important immunoregulatory role in the prevention of humoral autoimmunity through the elimination of both autoreactive B cells and ag-specific T cells. Moreover, an ineffective initial CTL response can evolve into a persistent antibody-mediated response and with it, the potential for sustained humoral autoimmunity.

L4 ANSWER 14 OF 37 MEDLINE

AN 1999218481 MEDLINE  
DN 99218481 PubMed ID 10201983  
TI Altered CD3- and CD28-mediated signaling events in cord blood T cells are associated with dysfunctional regulation of Fas ligand-mediated cytotoxicity.

AU Sato K, Nagayama H, Takahashi T A  
CS Department of Cell Processing, Institute of Medical Science, University of Tokyo, Japan  
SO JOURNAL OF IMMUNOLOGY (1999 Apr 15) 162 (8):4464-71  
Journal code IFB 2985117R ISSN 0022-1767

CY United States  
DT Journal Article, (JOURNAL ARTICLE)  
LA English  
FS Abridged Index Medicus Journals, Priority Journals  
EM 199905  
ED Entered STN 19990517  
Last Updated on STN 19990517

AB There have been numerous reports of decreased acute and chronic graft-versus-host disease (GVHD) in patients receiving HLA-matched grafts or HLA-disparate umbilical cord transplants. However, little is known about the mechanisms underlying the low incidence of GVHD in umbilical cord blood transplantation (CBT). In this study, we examined CD3- and CD28-mediated functional properties and signaling events in CBT cells (CBTCs). Dual stimulation of peripheral blood TCs (PBTCs) and bone marrow TCs (BMTCs) with mAbs to CD3- and CD28-induced expressions of Fas ligand (FasL), as well as CD25 and CD134 (CD134L), whereas defective induction of these activation-associated cell surface molecules were observed in CBTCs. Engagement of both CD3 and CD28

induced FasL-mediated cytotoxicity in peripheral blood TCs (PBTCs) but not CBTCs, however, both of these tissue sources possess intrinsically similar proliferative responsiveness. Analysis of CD3- and CD28-induced signal transduction revealed a deficiency in signaling events that involved repressed tyrosine phosphorylation and enzymatic activities of a family of

mitogen-activated protein kinases, extracellular signal-regulated kinase 2, stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and p38mapk, as well as p56lck and ZAP-70 in CBTCs compared with those in PBTCs. These results suggest that CD3- and CD28-mediated signaling events blockage in CBTCs may be responsible for dysfunction of FasL-mediated cytotoxicity and lead to the low incidence of severe GVHD in CBT.

L4 ANSWER 15 OF 37 MEDLINE DUPLICATE 9  
AN 1999211960 MEDLINE  
DN 99211960 PubMed ID 10194454

TI Graft-versus-leukemia effect and graft-versus-host disease can be differentiated by cytokine mechanisms in a

- murine model of allogeneic bone marrow transplantation  
AU Tsukada N, Kobata T, Aizawa Y, Yagita H, Okumura K  
CS Department of Immunology, Juntendo University School of Medicine,  
Tokyo, Japan  
SO BLOOD (1998 Apr 15) 93 (8) 2738-47  
Journal code A85, 7803309 ISSN 0006-4971  
CY United States  
DT Journal Article (JOURNAL ARTICLE)  
LA English  
FS Abundant Index Medicus Journals, Priority Journals  
EM 199905  
ED Entered STN 19990517  
Last Updated on STN 19990517  
AB Allogeneic bone marrow transplantation (allo-BMT) is associated with both graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) effect. In the present study, we examined the contribution of cytokine effector mechanisms, which are mediated by tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Fas ligand (FasL), or perforin, to GVHD and GVL effect in a murine BMT model. Bone marrow cells plus spleen cells (BMS) from wild-type, FasL-defective, or perforin-deficient donors were transferred into lethally irradiated recipients in the parent (C57BL/6) to F1 (C57BL/6 x DBA/2) BMT model without prior inoculation of DBA/2 leukemia L1210 or P815 mast celloma cells. The effect of anti-TNF- $\alpha$  antibody administration was also examined. Whereas the defect or blockade of each cytokine pathway could ameliorate lethal acute GVHD, the GVL effect was differentially affected. The wild-type BMS recipients died of acute GVHD within 50 days without residual leukemia cells. The FasL-defective BMS recipients showed 80% survival over 80 days without acute GVHD or residual leukemia cells. Administration of anti-TNF- $\alpha$  antibody resulted in early leukemia relapse and the recipients died within 25 days with massive leukemia infiltration in the liver. The perforin-deficient BMS recipients died within 60 days with residual leukemia cells. These results suggest that blockade of the Fas/FasL pathway could be used for ameliorating GVHD without impairing GVL effect in allo-BMT
- L4 ANSWER 16 OF 37 MEDLINE  
AN 200000330 MEDLINE  
DN 20000330 PubMed ID 10532530  
TI Analysis of Fas system in pulmonary injury of graft-versus-host disease after rat intestinal transplantation  
AU Hiroyasu S, Shiraishi M, Koji T, Mamada T, Sugawa H, Tomon H, Muto Y  
CS The First Department of Surgery, University of the Ryukyus, School of Medicine, Okinawa, Japan  
SO TRANSPLANTATION (1998 Oct 15) 68 (7) 933-8  
Journal code WEI, 0132144 ISSN 0041-1337  
CY United States  
DT Journal Article (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199911  
ED Entered STN 20000111  
Last Updated on STN 20000111  
AB The lung is one of the primary targets of acute graft-versus-host disease (GVHD), which is the principal complication that occurs after allogeneic intestinal transplantation. The purpose of this study is to investigate the involvement of Fas/Fas ligand system in pulmonary injury after rat semi-allogeneic intestinal transplantation. The lungs were serially harvested from LEW x BN-F1(BLNF1) recipients of either LEW heterotopic intestinal allografts or BLNF1 isografts, on days 1, 3, 5, 9, and 13 posttransplant. In light microscopy, pulmonary injury became apparent on day 13 in the allogeneic combination, showing a thickening of the alveolar septa. The incidence of apoptosis, examined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) biotin nick end-labeling, was observed to increase steadily in the alveolar cells accompanied by a progression of GVHD in an immunohistochemical study. Fas was constitutively expressed in the lung, although Fas ligand was expressed most extensively on day 9. The immunoreactivity of both Fas and Fas ligand were observed in alveolar cells, in addition to leukocytes. An analysis by reverse transcription polymerase chain reaction also revealed that the expression of Fas mRNA was constitutive without any significant change, although that of Fas ligand mRNA increased substantially and peaked on day 9, which was significant compared to the isogenic combination. In conclusion, transcriptionally up-regulated Fas ligand and increased number of apoptotic suggests that the Fas system may play a role in the pathophysiology of GVHD-induced pulmonary injury
- L4 ANSWER 17 OF 37 MEDLINE DUPLICATE 10  
AN 1999289372 MEDLINE  
DN 99289372 PubMed ID 10360966  
TI Therapeutic effect of an anti-Fas ligand mAb on lethal graft-versus-host disease  
AU Miwa K, Hashimoto H, Yanoji T, Nakamura N, Nagata S, Suga T  
CS Department of Molecular Biology, Osaka Bioscience Institute, Osaka 565-0874, Japan  
SO INTERNATIONAL IMMUNOLOGY (1999 Jun) 11 (6) 925-31  
Journal code AVS, 8916182 ISSN 0953-8178  
CY ENGLAND United Kingdom  
DT Journal Article (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199908  
ED Entered STN 19990820  
Last Updated on STN 19990820  
AB Several anti-Fas ligand (FasL) inhibitory mAb (FLM) were raised and characterized in this study. One, FLM58, showed more potent neutralizing activity than Fas-Fc, the previously established artificial neutralizing agent for FasL. Several murine models of acute graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation have been used to show that both FasL and perforin, the major effector molecules of cytotoxic T lymphocytes, are involved in this disease. In our GVHD model, FasL, rather than perforin was associated with lethality. Administration of FLM58 or Fas-Fc reduced the weight loss and mortality caused by GVHD, although other signs of GVHD, such as skin lesions, lymphoid hypoplasia and mononuclear cell infiltration in the liver, did not improve significantly. FLM58 was more effective than Fas-Fc in reducing mortality. Our results demonstrated that neutralizing agents for FasL are therapeutic for lethal GVHD
- L4 ANSWER 18 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI B V DUPLICATE 11  
AN 1999380803 EMBASE  
TI Graft versus-host disease-like immunophenotype and apoptotic keratinocyte death in paraneoplastic pemphigus  
AU Reich K, Brinck U, Jatschert M, Blaschke V, Dames K, Braess J, Wormann B, Rungger T M, Neumann C  
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SO British Journal of Dermatology (1999) 141(4) 738-745  
Refs 34  
ISSN 0007-0963 CODEN BJDEAZ  
CY United Kingdom  
DT Journal Article  
FS 013 Dermatology and Venereology  
037 Drug Literature Index  
LA English  
ST English  
AB Paraneoplastic pemphigus (PP) is an autoimmune disease, which is frequently associated with non-Hodgkin's lymphoma. Autoantibodies against components of the cytoplasmic plaque of epithelial desmosomes are usually present in the sera and are believed to play a major pathogenic part in acantholysis and suprabasal epidermal blistering. However, another typical histological feature of PP, interface dermatitis with keratinocyte dyskeratosis, is shared with skin diseases that involve epithelial damage mediated by T cells. Here, we present the detailed characterization of the cutaneous T cell response in a patient with PP and demonstrate a selective epidermal accumulation of activated CD4+ T cells together with an increased local production of interferon- $\gamma$ , gamma, and tumor necrosis factor- $\alpha$ , alpha, and a strong expression of HLA-DR and ICAM-1 on keratinocytes. Apoptosis was identified as a key mechanism of keratinocyte death and appeared
- independent of the Fas/Fas ligand (FasL) pathway as epidermal expression of Fas was not increased compared with normal skin, and FasL was undetectable on the protein and mRNA level. Triple therapy with high-dose corticosteroids, cyclophosphamide and intravenous immunoglobulins reduced levels of pemphigus-like autoantibodies and reversed the cutaneous inflammatory reaction leading to long-standing clinical remission. Our findings support the concept of a major contribution of cytotoxic T lymphocytes to the immunopathology of paraneoplastic pemphigus
- L4 ANSWER 19 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)  
AN 1999 556331 SCISEARCH  
GA The Genuine Article (R) Number 215WW  
TI An in vitro model of allogeneic stimulation of cord blood: induction of Fas independent apoptosis  
AU El-Ghazouani A, Drenou B, Blanchetel V, Choquet C, Fauchet R, Charron D, Mooney N (Reprint)  
CS INST BIOMED CORDELIERS, INSERM, U396 IMMUNOGENET MOL LAB, 15 RUE ECOLE MED, F-75006 PARIS, FRANCE (Reprint), INST BIOMED CORDELIERS, INSERM, IMMUNOGENET MOL LAB, F-75006 PARIS, FRANCE, CHRU PONTCHAILLON LAB HEMATOLOGIE & IMMUNOLOGIE, F-33003 RENNES 9, FRANCE  
CYA FRANCE  
SO HUMAN IMMUNOLOGY (JUL 1999 Vol 60, No 7, pp. 598-607)  
Publisher ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010  
ISSN 0198-8859  
DT Article, Journal  
FS LIFE  
LA English  
REC Reference Count 30  
\*ABSTRACT IS AVAILABLE IN THE ALL AND ALL FORMATS\*  
AB Cord blood is increasingly used in transplantation as it is a readily available source of progenitor cells and is reputed to generate less severe graft-versus-host disease (GVHD) than adult bone marrow. We have compared apoptosis of cord blood lymphocytes (CB) and adult lymphocytes (PBMC) after stimulation via HLA class I, HLA class II or CD3 in order to reproduce in vitro some of the stimuli occurring after allotransplantation. CB spontaneously apoptose more than PBMC ex vivo, stimulation via HLA class I dramatically increased CB apoptosis without altering viability of PBMC. Expression of Fas was markedly lower on CB than on PBMC and this difference was maintained even after activation. Fas ligand was expressed in CB and in PBMC. CB were activated via either HLA class I or class II molecules although proliferation was not observed. Only phorbol ester pre-activation allowed Fas to subsequently induce a death signal. Proliferation of PBMC with regard to both spontaneous and activation induced apoptosis and either allo- or CD3 mediated stimulation. Finally, the apoptosis of CB via HLA class I could have an important role in the moderation of graft-versus-host disease. Human Immunology 60, 598-607 (1999) (C) American Society for Histocompatibility and Immunogenetics, 1999. Published by Elsevier Science Inc.
- L4 ANSWER 20 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)  
AN 1999 358258 SCISEARCH  
GA The Genuine Article (R) Number 192CP  
TI A metalloproteinase inhibitor prevents acute graft-versus-host disease while preserving the graft-versus-leukemia effect of allogeneic bone marrow transplantation  
AU Hattori K, Hirano T (Reprint), Miyajima H, Yamakawa N, Ikeda S, Yoshino K, Tateo M, Oshimi K, Karagaki N, Yagita H, Okumura K  
CS JUNTENDO UNIV, SCH MED, DEPT INTERNAL MED, DIV HAEMATOLOG, BUNKYO KU 2-1-1, JAPAN (Reprint), JUNTENDO UNIV, SCH MED, HONGO, TOKYO 113, JAPAN (Reprint), JUNTENDO UNIV, SCH MED, DEPT INTERNAL MED, DIV HAEMATOLOG, BUNKYO KU, TOKYO 113, JAPAN, JUNTENDO UNIV, SCH MED, DIV PATHOBIOLOG, TOKYO 113, JAPAN, JUNTENDO UNIV, SCH MED, DEPT IMMUNOL.

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HOKKAIDO JAPAN JAPAN  
SCI & TECHNOL CORP CREST TOKYO JAPAN

CYA JAPAN  
SO BRITISH JOURNAL OF HAEMATOLOGY (APR 1999) Vol 105, No 1, pp  
303-312

Publisher BLACKWELL SCIENCE LTD P O BOX 88, OSNEY MEAD  
OXFORD OX2 0NE  
OXON ENGLAND  
ISSN 0007-1048  
DT Article, Journal  
FS LIFE, CLIN  
LA English

REC Reference Count 39

ABSTRACTS IS AVAILABLE IN THE ALL AND ALL FORMATS.

We examined the effect of a hydroxamic acid-based matrix  
metalloproteinase inhibitor (KB-R7785), which we previously demonstrated  
to have a potent ameliorating effect on acute graft  
versus host disease (GVHD), and on the

graft-versus-leukemia (GVL) effect of allogeneic bone marrow  
transplantation (BMT). KB-R7785 was administered to (C57BL/6 x BALB/c)

CB1-1 mice that had been inoculated with IgE-producing B53 hybridoma  
cells of BALB/c origin as a model tumour, along with or without  
transplantation of C57BL/6 (B6) bone marrow cells and spleen cells (BMS).

Administration of KB-R7785 without EMS significantly prolonged the  
survival of B53-inoculated CB1-1 mice by inhibiting the infiltration of B53  
cells into the liver and spleen. Transplantation of B6 BMS without

KB-R7785 resulted in the death of most recipients due to acute  
GVHD while efficiently eliminating B53 cells. Administration of  
KB-R7785 along with B6 BMS resulted in a 50% survival of B53-inoculated  
CB1-1 mice over 50 d without histological manifestations of acute

GVHD or residual B53 cells. These results indicate the beneficial  
effects of KB-R7785 that inhibit tumour infiltration and prevent acute  
GVHD while preserving the GVL effect of allogeneic BMT.

ANSWER 21 OF 37 MEDLINE DUPLICATE 12

AN 1999332261 MEDLINE  
DN 99332261 PubMed ID 10403735

TI Intestinal crypt cell apoptosis in murine acute graft  
versus host disease is mediated by tumour necrosis

factor alpha and not by the Fas-Fas interaction: effect of pentoxifylline  
on the development of mucosal atrophy

AU Stuber E, Buscherfeld A, von Freier A, Arent T, Folser U R  
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SO GUT (1999 Aug) 45 (2) 229-35

Journal code EVT 2985108R ISSN 0017-5149

CY ENGLAND United Kingdom

DT Journal Article (JOURNAL ARTICLE)

EM 199908

ED Entered STN 19990825

Last Updated on STN 19990825

AB BACKGROUND: Murine T cell mediated acute semiallogeneic graft  
versus host disease (GVHD) is characterized by

lymphocytic infiltrates, crypt hyperplasia, and villous atrophy. It has  
been shown that programmed cell death (apoptosis) of the crypt epithelium  
takes place during the intestinal manifestation of acute GVHD.

AIMS: To investigate which of the two most investigated inducers of  
apoptosis, Fas ligand (FasL), and tumour necrosis  
factor alpha (TNF-alpha) is responsible for the induction of apoptosis in  
this animal model. METHODS: Animals undergoing acute semiallogeneic

reaction were treated with neutralising anti-TNF-alpha. Two different  
anti-FasL antibodies, or pentoxifylline. RESULTS: Anti-TNF-alpha  
application inhibited the appearance of apoptotic cells in the intestinal  
mucosa, whereas anti-FasL antibodies had no influence on mucosal  
apoptosis. In addition, the transfer of FasL-deficient (gld) donor  
lymphocytes still induced crypt cell apoptosis, villous atrophy, and crypt  
hyperplasia. Furthermore, when the animals were treated with  
pentoxifylline, a known inhibitor of TNF-alpha secretion in vitro and in

vivo, there was significant normalisation of the intestinal morphology  
accompanied by inhibition of epithelial apoptosis. CONCLUSIONS: The  
FasL-Fas interaction is not involved in the induction of apoptosis during  
acute GVHD. However, neutralisation of TNF-alpha by an  
antibody or by pentoxifylline inhibits the occurrence of apoptosis  
and of mucosal atrophy in this animal model. These results have  
implications for the treatment of immunologically mediated human atrophic  
gut diseases, for example, diet-related cases of coeliac disease.

ANSWER 22 OF 37 MEDLINE DUPLICATE 13

AN 1999239157 MEDLINE  
DN 99239157 PubMed ID 10222651

TI Therapeutic strategy for post-transfusion graft-vs. host disease

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SO INTERNATIONAL JOURNAL OF HAEMATOLOGY (1999 Apr) 89 (3) 147-51

Journal code AFE 911162Z ISSN 0935-5710

CY Ireland

DT Journal Article (JOURNAL ARTICLE)

LA English

EM 199905

ED Entered STN 19990601

Last Updated on STN 19990601

AB An effective treatment for post-transfusion graft-vs. host disease (PT-  
GVHD), a fatal complication of blood transfusion, has not yet been  
identified. In this review, we propose a treatment for PT-GVHD  
based on the mechanism of its onset. First, we briefly review the findings  
that PT-GVHD is induced by cytotoxic T-lymphocyte (CTL)-mediated  
tissue injuries through the Fas/Fas ligand system, the  
perforin/granzyme system, and alloantigen-specific antibodies  
as well as through inflammatory cytokines. Secondly, we emphasize the  
usefulness of a serine protease inhibitor for the inhibition of  
CTL-mediated cytotoxicity in the earlier stages of onset. Subsequent  
administration of methylprednisolone and 2-chlorodeoxyadenosine is  
recommended for elimination of the donor's lymphocytes. The usefulness of  
chloroquine for the suppression of CTL activity and the production of  
tumour necrosis factor as well as the efficiency of pentoxifylline for the  
suppression of the production of tumour necrosis factor are also discussed.  
Therapeutic strategies for PT-GVHD should also be useful for  
treating acute GVHD secondary to allogeneic bone marrow  
transplantation, and to prevent the host's rejection of transplanted  
organs as well as tissue damage in autoimmune diseases.

ANSWER 23 OF 37 MEDLINE DUPLICATE 14

AN 1999321949 MEDLINE  
DN 99321949 PubMed ID 10393698

TI Active participation of CCR5(+)CD8(+) T lymphocytes in the pathogenesis of  
liver injury in graft-versus-host disease

AU Murai M, Yoneyama H, Harada A, Yi Z, Vestergaard C, Guo B, Suzuki K,  
Asakura H, Matsushima K

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SO JOURNAL OF CLINICAL INVESTIGATION (1999 Jul) 104 (1) 49-57

Journal code HST 7802877 ISSN 0021-9738

CY United States

DT Journal Article (JOURNAL ARTICLE)

LA English

FS Abstracted Index Medicus Journals, Priority Journals

EM 199908

ED Entered STN 19990816

Last Updated on STN 19990816

AB We examined the molecular pathogenesis of graft-versus  
host disease-associated (GVHD-associated) liver  
injury in mice, focusing on the role of chemokines. At the second week  
after cell transfer in the patient-intra-F-1 model of GVHD, CD8(+) T  
cells - especially donor-derived CD8(+) T cells - infiltrated the  
liver, causing both portal hepatitis and non-suppurative destructive  
cholangitis (NSDC). These migrating cells expressed CCR5. Moreover,

macrophage inflammatory protein-1alpha (MIP-1alpha), one of the ligands  
for CCR5, was selectively expressed on intrahepatic bile duct epithelial  
cells, endothelial cells, and infiltrating macrophages and lymphocytes.  
Administration of anti-CCR5 antibody dramatically reduced the  
infiltration of CCR5(+)CD8(+) T lymphocytes into the liver, and  
consequently protected against liver damage in GVHD. The levels  
of Fas ligand (FasL) mRNA expression in the liver were  
also decreased by anti-CCR5 antibody treatment. Anti-MIP-1alpha  
antibody treatment also reduced liver injury. These results  
suggest that MIP-1alpha-induced migration of CCR5-expressing CD8(+) T  
cells into the portal areas of the liver plays a significant role in  
causing liver injury in GVHD, thus CCR5 and its ligand may be  
the novel target molecules of therapeutic intervention of hepatic  
GVHD.

ANSWER 24 OF 37 MEDLINE

AN 2000258609 MEDLINE  
DN 20025809 PubMed ID 10555993

TI Effect of graft-versus-host disease (GVHD) on host hematopoietic progenitor cells is mediated by Fas-  
Fas ligand interactions but this does not explain the  
effect of GVHD on donor cells

AU Iwasaki T, Hamano T, Saitoh K, Kuwano T, Katsuka Y, Takemoto Y, Ogata  
A, Sugihara A, Terada N, Fujimoto J, Kakihara E

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First Department of Surgery, Hyogo College of Medicine, 1-1 Mikogawa-cho,  
Nishinomiya, Hyogo 663-8501, Japan

SO CELLULAR IMMUNOLOGY (1999 Oct 10) 197 (1) 30-8

Journal code CG9 126405 ISSN 0008-8749

CY United States

DT Journal Article (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 200001

ED Entered STN 20000114

Last Updated on STN 20000114

AB The acute graft-versus-host disease (GVHD) generated in BDF1 mice by the injection of spleen cells from  
the C57BL/6 parental strain induces a direct cell-mediated attack on host  
lymphohematopoietic populations, resulting in the reconstitution of the  
host with donor hematopoietic stem cells. We examined the effect of  
GVHD on the donor and host hematopoiesis in parental induced acute  
GVHD. The bone marrow was hypoplastic and the number of  
hematopoietic progenitor cells significantly decreased at 4 weeks after  
GVHD induction. However, extramedullary splenic hematopoiesis was  
present and the number of hematopoietic progenitor cells in the spleen  
significantly increased at this time. Fas expression on the host spleen  
cells and bone marrow cells significantly increased during weeks 2 to 8 of  
GVHD. Host cell incubation with anti-Fas Ab induced apoptosis, and  
the number of hematopoietic progenitor cells decreased during these weeks.  
A significant correlation between the augmented Fas expression on host  
bone marrow cells and the decreased number of host bone marrow cells by  
acute GVHD was observed. Furthermore, the injection of  
Fas ligand (FasL)-deficient B6gld spleen cells failed  
to affect host bone marrow cells. Although Fas expression on repopulating  
donor cells also increased, Fas-induced apoptosis by the repopulating  
donor cells was not remarkable until 12 weeks, when more than 90% of the  
cells were donor cells. The number of hematopoietic progenitor cells in  
the bone marrow and the spleen by the repopulating donor cells, however,  
decreased over an extended time during acute GVHD. This suggests  
that Fas-FasL interactions may regulate suppression of host hematopoietic  
cells but not of donor hematopoietic cells. Hematopoietic dysfunctions  
caused by the reconstituted donor cells are independent to Fas-FasL  
interactions and persisted for a long time during parental-induced acute  
GVHD.

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ANSWER 25 OF 37 MEDLINE DUPLICATE 15

AN 1998261428 MEDLINE  
DN 98261428 PubMed ID 9596649

TI Differential effects of anti-Fas ligand and anti-tumor  
necrosis factor alpha antibodies on acute graft-  
versus-host disease pathologies

In addition to these immunosuppressive agents, monoclonal antibodies against functional cell surface molecules, such as LFA-1, ICAM-1, Fas and Fas ligand, must be effective for treatment of TA. GVHD. Since the effective standard therapy of TA, GVHD has not been established, the prevention by gamma irradiation of cellular blood components is most important.

L4 ANSWER 31 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)  
AN 97 877846 SCISEARCH  
GA The Genuine Article (R) Number YG424  
TI The synergistic effects of anti-Fas ligand and TNF-alpha antibody on the prevention of lethal acute graft-versus-host disease in mice  
AU Hattori K, Hirano T, Tatemoto M, Oshimi K, Kayagaki N, Yagita H, Okumura K  
CS JUNTENDO UNIV, SCH MED, SAPPORO CITY GEN HOSP, DEPT PATHOL, TOKYO 113  
JAPAN, JUNTENDO UNIV, SCH MED, SAPPORO CITY GEN HOSP, DEPT INTERNAL MED  
DIV HEMATOL, TOKYO 113, JAPAN, JUNTENDO UNIV, SCH MED, SAPPORO CITY GEN HOSP, DEPT IMMUNOL, TOKYO 113, JAPAN  
CVA JAPAN  
BLOOD (15 NOV 1997) Vol. 90, No. 10, Part 1, Supp. [1], pp. 907-907  
Publisher: W.B. SAUNDERS CO., INDEPENDENCE SQUARE WEST  
300 PHILADELPHIA, PA 19106-3399  
ISSN 0006-4971  
DT Conference, Journal  
FS LIFE CLIN  
LA English  
REC Reference Count: 0

L4 ANSWER 32 OF 37 MEDLINE  
AN 1998062247 MEDLINE  
DN 98062247 Pubmed ID 9407075  
TI Activity of TNF-related apoptosis-inducing ligand (TRAIL) in haematological malignancies  
AU Snell V, Clot K, Zuo S, Goodwin R, Thomas E K, Morris S W, Kadim M E, Cabanillas F, Andrieu M, Younes A  
CS Department of Hematology, University of Texas M D Anderson Cancer Center, Houston 77030 USA  
NC CA 01702 (NCI)  
CA 21765 (NCI)  
CA 68129 (NCI)  
SD BRITISH JOURNAL OF HAEMATOLOGY (1997 Dec) 99 (3) 618-24  
Journal code AXC: 0372544 ISSN 0007-1048  
CY ENGLAND, United Kingdom  
DT Journal Article (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
TM 199801  
Entered STN 19980206  
Last Updated on STN 19980206  
Entered Medicine 19980123

AB T-cell cytotoxicity is primarily mediated by two cell surface proteins, Fas ligand (FasL) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), and intracellular perforin and granzyme granules. FasL-deficient and perforin-deficient T lymphocytes maintain cytotoxicity but fail to induce graft-versus-host disease (GVHD) when transplanted into mice suggesting that GVHD and graft-versus-tumour (GVT) effects can be dissociated, and that TRAIL is not involved in the pathogenesis of GVHD. Because TRAIL could mediate a favourable GVT effect, it became important to study the spectrum of its activity and to investigate factors that can dissociate its expression from FasL. TRAIL induced apoptosis in 11/41 (27%) tumour specimens of haematological origin compared to 16/41 (39%) induced by FasL. Although eight specimens were sensitive to both FasL and TRAIL, no synergism was observed between these

two ligands. TRAIL induced apoptosis in a dose and time dependent manner with an ED50 of 0.5 microg/ml and EDmax of 1 microg/ml. TRAIL activity was not reduced by the over-expression of the inhibiting resistant (MDR) protein, and was not enhanced by 8-cis retinoic acid (RA). Both ligands were simultaneously up-regulated in normal peripheral blood lymphocytes in response to IL-2, IL-15 and

anti-CD3 antibody whereas IL-10 had no effect. Together, our data show that (1) TRAIL can mediate cell death in a variety of human haematological malignancies; (2) resistance to TRAIL is not mediated by MDR protein; (3) the lack of synergy between TRAIL and FasL suggests that either one is sufficient to mediate T-cell cytotoxicity; and (4) within the panel of cytokines tested, the expression of TRAIL and FasL could not be dissociated.

L4 ANSWER 33 OF 37 MEDLINE  
AN 97369712 MEDLINE  
DN 97369712 Pubmed ID 9226153  
TI A metalloproteinase inhibitor prevents lethal acute graft-versus-host disease in mice  
AU Hattori K, Hirano T, Ushiyama C, Miyajima H, Yamakawa N, Ebata T, Wada Y, Ikeda S, Yoshino K, Tatemoto M, Oshimi K, Kayagaki N, Yagita H, Okumura K  
CS Department of Internal Medicine, Juntendo University School of Medicine, Bunkyo-ku, Tokyo, Japan  
BLOOD (1997 Jul 15) 90 (2) 542-8  
Journal code A8C: 7603509 ISSN 0006-4971  
CY United States  
DT Journal Article (JOURNAL ARTICLE)  
LA English  
FS Abstracted Index Medicus Journals, Priority Journals  
EM 199708  
Entered STN 19970813  
Last Updated on STN 2000303  
Entered Medicine 19970807

AB Tumor necrosis factor (TNF) and Fas ligand (FasL) have been implicated in the pathogenesis of graft-versus-host disease (GVHD), which is a major complication after allogeneic bone marrow transplantation. We examined here the ameliorating effect of a metalloproteinase inhibitor (KB-R7785) that inhibits TNF-alpha and FasL release in a lethal acute GVHD model in mice. Administration of KB-R7785 into (BALB/c x C57BL/6) F1 that received C57BL/6 spleen cells markedly reduced the mortality and weight loss in association with minimal signs of GVHD pathology in the liver, intestine, and hematopoietic tissues. The ameliorating effect of KB-R7785 was superior to that of anti-TNF-alpha antibody. Our results suggest that KB-R7785 could be a potent therapeutic agent for GVHD.

L4 ANSWER 34 OF 37 CANCERLIT  
AN 97619028 CANCERLIT  
DN 97619028  
TI Monocytes from cytokine mobilized stem cell products induce T cell apoptosis which is mediated in part by Fas ligand  
(Meeting abstract)  
AU Tamir J, Singh R K, Agerto A, Ino K  
CS University of Nebraska Medical Center, Omaha NE  
SO Proc Annu Meet Am Assoc Cancer Res (1997) Vol. 38, pp. A235  
ISSN 0197-018X  
DT (MEETING ABSTRACTS)  
FS ICD8  
LA English  
EM 199709  
Entered STN 19970909

AB Cytokine mobilized peripheral blood stem cell (PSC) products have a high frequency of cells which inhibit T cell function. To determine the origin and mechanism of this activity, mobilized PSC products were fractionated by Percoll centrifugation and immunomagnetic bead. Monocytes, which constitute approximately 10% percent of the cells within mobilized PSC products were found to inhibit T cell function via apoptosis. These cells inhibit both autologous and allogeneic T cell responses to PHA, pokeweed mitogen, OKT-3, IL-2, and tetanus toxin. The monocytes are low density, adherent and phagocytic, and are CD14+, HLA-DR+, CD11a+, CD11b+,

CD86+ (7+), CD80 (B7-1), CD16+, and CD11a+. Cell-cell contact is required for inhibition and is not neutralized by antibodies against TNF-alpha. However, the monocytes have increased mRNA levels for IL-10, and TNF compared to normal PB. The inhibition of both autologous and allogeneic T cell proliferation was associated with apoptosis as measured by hypodiploidy and DNA fragmentation. Furthermore, some but not all of the inhibitory activity could be prevented with antibodies to the CD85-ligand. This immunoregulatory activity (via apoptosis) has potential to regulate immune recovery following myeloablative therapy and the removal of these cells from the PSC has therapeutic potential. Conversely,

monocytes with T cell inhibitory activity have clinical potential for the control of allogeneic graft-versus-host disease or solid organ graft rejection.

L4 ANSWER 35 OF 37 BIOSIS  
AN 1998 67216 BIOSIS  
DN PREVIEW9800057216  
TI The synergistic effects of anti-Fas ligand and TNF-alpha antibody on the prevention of lethal acute graft-versus-host disease in mice  
AU Hattori K, Hirano T, Tatemoto M, Oshimi K, Kayagaki N, Yagita H, Okumura K  
CS (1) Div Hematol, Dep Internal Med, Juntendo Univ, Sch Med, Tokyo, Japan  
BLOOD (Nov 15, 1997) Vol. 90, No. 10 SUPPL. 1 PART 1, pp. 206A  
Meeting Info: 39th Annual Meeting of the American Society of Hematology, San Diego, California, USA December 5-9, 1997 The American Society of Hematology  
ISSN 0006-4971  
DT Conference  
LA English

L4 ANSWER 36 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)  
AN 97 524588 SCISEARCH  
GA The Genuine Article (R) Number XJ383  
TI Fas-mediated cytotoxicity by intestinal intraepithelial lymphocytes during acute graft-versus-host disease in mice  
AU Sakai T, Kimura Y, Inagaki-Obara K, Kusugami K, Lynch D H, Yoshitaka Y  
CS NAGOYA UNIV, SCH MED, DIS MECHANISM & CONTROL RES INST, LAB GERMFREE LIFE, SHOWA KU, 65 TSURUMAI CHO, NAGOYA, AICHI 468, JAPAN (Reprint), NAGOYA UNIV, SCH MED, DEPT INTERNAL MED 1, NAGOYA, AICHI 468, JAPAN, IMMUNE CORP, DEPT IMMUNOL, SEATTLE, WA  
CVA JAPAN, USA  
SO GASTROENTEROLOGY (JUL 1997) Vol. 113, No. 1, pp. 168-174  
Publisher: W.B. SAUNDERS CO., INDEPENDENCE SQUARE WEST  
300 PHILADELPHIA, PA 19106-3399  
ISSN 0016-5085  
DT Article, Journal  
FS LIFE CLIN  
LA English  
REC Reference Count: 35

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*  
AB Background & Aims: Host-derived intestinal intraepithelial lymphocytes (IELs) increase in number during acute graft-versus-host disease (GVHD) in mice. In the present study, we examined Fas-mediated cytotoxicity by host-derived IELs against Fas-expressing target cells to see whether Fas/Fas ligand (Fas-L) interaction is involved in the pathogenesis of enteropathy during acute GVHD. Methods: Acute GVHD was induced by injection of parental spleen cells into nonirradiated F1 mice. The expression of Fas antigen on the intestinal epithelial cells (IECs) was examined by flow cytometry, and the expression of messenger RNA (mRNA)

for Fas-L, interleukin 2, and interferon gamma in host-derived IELs was assessed by reverse-transcription polymerase chain reaction. Fas-mediated cytotoxicity by host-derived IELs was assessed using Fas-transfected cells, IECs, and Fas immunoglobulin Fe fusion protein (Fas-Fc). Results: Fas antigen was constitutively expressed on the cell surface of IECs before and after GVHD induction. Although Fas-L mRNA was not detected or detected scarcely in either alpha beta or gamma delta IELs before GVHD induction, both IELs expressed high levels of mRNA for Fas-L and interferon gamma after GVHD induction.

Host-derived IELs during acute GVHD showed cytotoxicity against Fas-transfected target cells and IECs, which was partly blocked by addition of Fas-Fc. Conclusions: Fas/FasL-mediated cytotoxicity by host-derived IELs may be partly responsible for the enteropathy during acute GVHD.

L4 ANSWER 37 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)  
AN 95 42431 SCISEARCH  
GA The Genuine Article (R) Number GQ402

L4 ANSWER 38 OF 37 MEDLINE  
AN 97369712 MEDLINE  
DN 97369712 Pubmed ID 9226153  
TI A metalloproteinase inhibitor prevents lethal acute graft-versus-host disease in mice  
AU Hattori K, Hirano T, Ushiyama C, Miyajima H, Yamakawa N, Ebata T, Wada Y, Ikeda S, Yoshino K, Tatemoto M, Oshimi K, Kayagaki N, Yagita H, Okumura K  
CS Department of Internal Medicine, Juntendo University School of Medicine, Bunkyo-ku, Tokyo, Japan  
BLOOD (1997 Jul 15) 90 (2) 542-8  
Journal code A8C: 7603509 ISSN 0006-4971  
CY United States  
DT Journal Article (JOURNAL ARTICLE)  
LA English  
FS Abstracted Index Medicus Journals, Priority Journals  
EM 199708  
Entered STN 19970813  
Last Updated on STN 2000303  
Entered Medicine 19970807

AB Tumor necrosis factor (TNF) and Fas ligand (FasL) have been implicated in the pathogenesis of graft-versus-host disease (GVHD), which is a major complication after allogeneic bone marrow transplantation. We examined here the ameliorating effect of a metalloproteinase inhibitor (KB-R7785) that inhibits TNF-alpha and FasL release in a lethal acute GVHD model in mice. Administration of KB-R7785 into (BALB/c x C57BL/6) F1 that received C57BL/6 spleen cells markedly reduced the mortality and weight loss in association with minimal signs of GVHD pathology in the liver, intestine, and hematopoietic tissues. The ameliorating effect of KB-R7785 was superior to that of anti-TNF-alpha antibody. Our results suggest that KB-R7785 could be a potent therapeutic agent for GVHD.

L4 ANSWER 39 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)  
AN 97 524588 SCISEARCH  
GA The Genuine Article (R) Number XJ383

11. MASSIVE UP-REGULATION OF THE FAS LIGAND IN LIVER AND GLID  
MICE - IMPLICATIONS FOR FAS REGULATION AND THE GRAFT-  
VERSUS-HOST DISEASE-LIKE WASTING SYNDROME  
AU CHU J L, RAMOS P, ROSENDORFF A, NIKOLICZUKIC J, LACV E,  
MATSUZAWA A, ELKON K B (Reprint)  
CORNELL UNIV, HOSP SPECIAL SURG, MED CTR, SPECIALIZED CTR  
RES SYST LUPUS  
ERYTHEMATOSUS, NEW YORK, NY 10021 (Reprint), CORNELL UNIV,  
HOSP SPECIAL SURG, MED CTR, SPECIALIZED CTR RES SYST LUPUS  
ERYTHEMATOSUS, NEW YORK, NY  
10021 NEW SLOAN KETTERING CANC CTR, PROGRAM MOLEC BIOL,  
NEW YORK, NY  
10021 TOKAI UNIV, INST MED SCI, DEPT IMMUNOL, TOKYO 108, JAPAN,  
TOKAI  
UNIV, INST MED SCI, DEPT INTERNAL MED, TOKYO 108, JAPAN  
CYA USA, JAPAN  
SO JOURNAL OF EXPERIMENTAL MEDICINE, 101 JAN 1995 Vol 181, No 1,  
pp 393-398  
ISSN 0022-1007  
DT Note, Journal  
ENGLISH  
C. Reference Count 29  
\*ABSTRACT IS AVAILABLE IN THE ALL AND ALL FORMATS\*  
AB Fas-deficient lpr and gld mice develop lymphadenopathy due to the  
accumulation of T cells with an unusual double negative (DN)  
[CD4(-)CD8(-)] phenotype. Previous studies have shown that these abnormal  
cells are capable of inducing reduced lysis of certain Tc  
receptor-positive target cells. Since the Fas ligand  
(FasL) has recently been shown to be partly responsible for T  
cell-mediated cytotoxicity, lymph node cells from lpr and gld mice were  
examined for the expression of FasL mRNA. Northern blot analysis revealed  
that lymph node cells obtained from lpr and gld mice had a striking  
increase in the level of expression of FasL mRNA, predominantly due to  
expression in the DN T cells. Furthermore, lpr but not gld lymph node  
cells killed the B cell line, A20, in a Fas-dependent manner. These  
findings indicate that Fas mutations result in a massive up-regulation of  
FasL which, most likely, results from repetitive exposure to (self)  
antigen. This phenomenon could explain the lpr-induced wasting syndrome  
observed when lpr bone marrow-derived cells are adoptively transferred to  
wild type recipients

==> file uspatfull  
COST IN U S DOLLARS  
ENTRY SINCE FILE TOTAL  
SESSION  
FULL ESTIMATED COST 65 15 65 30

E:USPATFULL: ENTERED AT 10 13 02 ON 25 MAY 2001  
A INDEXING COPYRIGHT (C) 2001 AMERICAN CHEMICAL SOCIETY (ACS)  
FILE COVERS 1971 TO PATENT PUBLICATION DATE 22 MAY 2001  
(20010522/PD)  
FILE LAST UPDATED 22 MAY 2001 (20010522/E)  
HIGHEST PATENT NUMBER US6237146  
CA INDEXING IS CURRENT THROUGH 22 MAY 2001 (20010522/UPCA)  
ISSUE CLASS FIELDS (INCL) CURRENT THROUGH 22 MAY 2001  
(20010522/PD)  
REVISED CLASS FIELDS (INCL) LAST RELEASED APR 2001  
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE APR 2001

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>>> is included in file records. A thesaurus is available for the <<<  
>>> USPTO Manual of Classifications in the INCL, IFNCL, and R/PCL <<<  
>>> fields. This thesaurus includes catchword terms from the <<<  
>>> USPTO/MOC subject headings and subheadings. Thesaur are also <<<  
>>> available for the WPC International Patent Classification <<<  
>>> (IPC) Manuals, editions 1-5 in the I/CI, I/C2, I/C3, I/C4 <<<

>>> I/C5 and I/C (I/C6) fields, respectively. The thesaur in <<<  
>>> the I/C5 and I/C fields include the corresponding catchword <<<  
>>> terms from the IPC subject headings and subheadings <<<  
This file contains CAS Registry Numbers for easy and accurate  
substance identification

>> s 12 and (graft versus host or gvh)  
1099 FAS  
30218 LIGAND  
112 FAS LIGAND  
(FAS/LIGAND)  
48222 ANTIBODY  
25130 MONOCLON?  
31666 GRAFT  
112653 VERSUS  
96644 HOST  
1632 GRAFT VERSUS HOST  
(GRAFT(V)VERSUS(W)HOST)  
351 GVHD  
L5 17 L2 AND (GRAFT VERSUS HOST OR GVHD)  
==> d 15 btb ab

L5 ANSWER 1 OF 17 USPATFULL  
AN 2001 44200 USPATFULL  
TI Member of the TNF family useful for treatment and diagnosis of disease  
IN Wiley, Steven R., Libertyville, IL, United States  
PI Abbott Laboratories, Abbott Park, IL, United States (U S corporation)  
AU US 6207542, 20010327  
AI US 1998-10543, 19990626 (9)  
RL Continuation-in-part of Ser. No. US 1998-21706, filed on 10 Feb 1998,  
now abandoned Continuation-in-part of Ser. No. US 1997-798692, filed on  
12 Feb 1997, now abandoned  
DT Utility  
EXNAM Primary Examiner: Romero, David  
LREP Becker, Cheryl L., Goller, Mimi C.  
CLMN Number of Claims 2  
ECL Exemplary Claim 1  
DRWN 14 Drawing Figure(s) 9 Drawing Page(s)  
LN CNT 4355  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB An isolated clone consisting of sequences transcribed from the TREPA  
gene. Also provided are human polypeptides translated from said TREPA  
sequences and a procedure for producing such polypeptide by recombinant  
techniques. Also provided are a procedure for producing soluble  
biologically active TREPA, which may be used to treat deficiencies of  
TREPA and diseases conditions ameliorated by TREPA. Antibodies  
antagonists and inhibitors of sucr polypeptide which may be used to  
prevent the action of sucr polypeptide and therefore may be used  
therapeutically to treat TREPA-associated diseases, tumors or  
metastases are disclosed. Also disclosed is the use of said  
antibodies, agonists and inhibitors as well as the nucleic acid  
sequences to screen for, diagnose, prognosticate, stage and monitor  
conditions and diseases attributable to TREPA, especially inflammation  
The use of said partial sequence to provide antibodies  
agonists and inhibitors as well as partial nucleic acid sequences to  
screen for, diagnose, stage and monitor diseases associated with TREPA,  
including but not limited to inflammation, illustrative sequences and  
clone designations for TREPA are provided

>> d 15 2-17 btb ab

L5 ANSWER 2 OF 17 USPATFULL  
AN 2001 40270 USPATFULL  
TI Antisense inhibition of Fas mediated signaling  
IN Dean, Nicholas W., Oliverman, CA, United States  
Marcussen, Eric G., San Diego, CA, United States  
PA Isis Pharmaceuticals, Inc., Carlsbad, CA, United States (U S  
corporation)  
PI US 6204055, 20010320  
AI US 1999-290640, 19990412 (9)  
DT Utility

EXNAM Primary Examiner: Schwartzman, Robert A., Assistant Examiner  
Lacourcie, Karen A  
LREP Law Offices of Jamie Massey, Lucita  
CLMN Number of Claims 39  
ECL Exemplary Claim 1  
DRWN No Drawings  
LN CNT 1930  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB Compounds, compositions and methods are provided for inhibiting Fas  
mediated signaling. The compositions comprise antisense compounds  
targeted to nucleic acids encoding Fas, FasL and Fas-1. Methods of using  
these antisense compounds for inhibition of Fas, FasL and Fas-1  
expression and for treatment of diseases, particularly autoimmune and  
inflammatory diseases and cancers, associated with overexpression or  
constitutive activation of Fas, FasL or Fas-1 are provided

L5 ANSWER 3 OF 17 USPATFULL  
AN 2001 25915 USPATFULL  
TI Formamide compounds as therapeutic agents  
IN Andrews, Robert Carl, Durham, NC, United States  
Andersen, Marc Werner, Raleigh, NC, United States  
Butatz, Dulce Garrodo, Cary, NC, United States  
Chan, Joseph Hwang, Chapel Hill, NC, United States  
Cowan, David John, Hillsborough, NC, United States  
Gaul, Michael David, Apex, NC, United States  
McDougal, Daryl Lynn, Durham, NC, United States  
Musso, David Lee, Raleigh, NC, United States  
Rabinowitz, Michael Howard, Durham, NC, United States  
Santford, Jennifer Badang, Cary, NC, United States  
Wiethe, Robert William, Durham, NC, United States  
PA Glaxo Wellcome Inc., Research Triangle Park, NC, United States (U S  
corporation)  
PI US 6191150, 20010220  
AI US 1999-382747, 19990825 (9)  
PRAI GB 1998-18605, 19990826  
US 1998-97959, 19990826 (9)  
DT Utility  
EXNAM Primary Examiner: Davis, Zanna Northington, Assistant Examiner  
Robinson, Binta  
LREP Lemaitowicz, John L  
CLMN Number of Claims 19  
ECL Exemplary Claim 1  
DRWN No Drawings  
LN CNT 2829  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB A family of compounds having the general structural formula ##STR1##  
where W is a reverse hydroxamic acid group, and R sub 1, R sub 2,  
R sub 3, R sub 4, R sub 5 and R sub 6 are as described in the  
specification, or a pharmaceutically acceptable salt, sulfate,  
biologically ester, biologically amide, affinity reagent, or  
prodrug thereof

L5 ANSWER 4 OF 17 USPATFULL  
AN 2001 4739 USPATFULL  
TI Formamides as therapeutic agents  
IN Andrews, Robert Carl, Durham, NC, United States  
Andersen, Marc Werner, Raleigh, NC, United States  
Cowan, David John, Hillsborough, NC, United States  
Deaton, David Norman, Cary, NC, United States  
Dickerson, Scott Howard, Chapel Hill, NC, United States  
Dewery, David Harold, Durham, NC, United States  
Gaul, Michael David, Apex, NC, United States  
Luzzio, Michael Joseph, Durham, NC, United States  
Marron, Brian Edward, Durham, NC, United States  
Rabinowitz, Michael Howard, Durham, NC, United States  
PA Glaxo Wellcome Inc., Research Triangle Park, NC, United States (U S  
corporation)  
PI US 6172004, 20010109  
AI US 1999-382333, 19990825 (9)  
PRAI US 1998-97956, 19990826 (9)  
DT Patent  
EXNAM Primary Examiner: Lambkin, Deborah C  
LREP Lemaitowicz, John L  
CLMN Number of Claims 21  
ECL Exemplary Claim 1  
DRWN No Drawings

IN CNT 3155  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AR A family of compounds having the general structural formula ##STR1##

US ANSWER 5 OF 17 USPATFULL  
L5  
AN 2000 167719 USPATFULL  
T1 Dao, a Fas-binding protein that activates JNK and apoptosis  
IN Yang, Xiaoli, Philadelphia, PA, United States  
Khosravi-Far, Roya, Malden, MA, United States  
Chang, Howard Y, Cambridge, MA, United States  
PA Baltimore, David, Pasadena, CA, United States  
(U.S. coporation)  
P1 US 6159731 200001212  
A1 US 1998-22883 19980212 (9)  
PRA1 US 1997-37919 19970212 (60)  
US 1997-51753 19970626 (60)

LREP Wolf, Greenfield & Sacks P.C.  
 CLMN Number of Claims 14  
 ECL Exemplary Claim 1  
 DRWN 10 Drawing Figure(s), 9 Drawing Page(s)  
 LN CNT 2773  
 CAS INDEXING IS AVAILABLE FOR THIS PATENT  
 AB The invention describes nucleic acids encoding the Daxx protein, including fragments and biologically functional variants thereof. Also included are polypeptides and fragments thereof encoded by such nucleic acids, and antibodies relating thereto. Methods and products for using such nucleic acids and polypeptides also are provided

L5 ANSWER 6 OF 17 USPATFULL  
AN 2000 160799 USPATFULL  
T1 Death domain containing receptors  
IN Yu, Guo-Liang, Darrestrom, MD, United States  
N1 Jian, Rockville, MD, United States  
Gentz, Reimer L, Silver Spring, MD, United States  
Dillon, Patrick J, Gaithersburg, MD, United States  
PA Human Genome Sciences, Inc., Rockville, MD, United States (U S  
corporation)  
P1 US 6153402, 2000111731  
A1 US 1997.815469, 199709121 (8)  
AN 1996.13285, 199603121 (60)  
US 1996.28711, 1996.1017 (60)  
US 1997.37341, 1997.0206 (60)

(TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR3 and DR3-V1 proteins. DR3 and DR3-V1 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR3 and DR3-V1 activity.

L5 ANSWER 7 OF 17 USPATFULL  
AN 2000 150164 USPATFULL  
TI Sulfamide-metalloprotease inhibitors

IN  
Bioeka Chris Allen Foster City, CA, United States  
Campbell Jeffrey Allen Fremont, CA, United States  
Castellano Airindo Lucas New City, NY, United States  
Chen Jian Jeffrey Santa Clara CA, United States  
Hendricks Robert Tian, Palo Alto, CA, United States  
Melnick Michael Joseph San Diego, CA, United States  
Walker Keith Adrian Murray, Los Altos Hills, CA, United States  
PA Sprintx (U.S.A.) Inc. Palo Alto, CA, United States (U.S. corporation)  
Agouron Pharmaceuticals, Inc., San Diego, CA, United States (U.S. corporation)  
PI US 6143744 2000/11/07  
AI US 1999-369501 1999/08/05 (9)  
RI Division of Ser. No. US 1998-3951, filed on 21 Jan 1998, now patented.  
Pat. No. US 59988412  
PRAL US 1987-36714 1997/07/23 (60)  
US 1997-52209 1997/10/16 (60)  
DT Utility  
EXNAM Primary Examiner: Raymond, Richard L  
LREP Peres, Rohan Bansal, Rekha  
CLMN Number of Claims 46  
ECL Exemplary Claim 1  
DRAWN No Drawings  
INCNT 1789  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB This invention relates to sulfamides of formula (I) ##STR1## that are inhibitors of metalloproteases, pharmaceutical compositions containing them, methods for their use and methods for preparing these compounds

L5 ANSWER 8 OF 17 USPTA/FULL  
AN 2000 134887 USPTA/FULL  
T1 Sulfamide-metalloproteinase inhibitors  
IN Bicka, Chris Allen, Foster City, CA, United States  
Campbell, Jeffrey Allen, Fremont, CA, United States  
Castellano, Alimdo Lucas, New Cht., NY, United States  
Chen, Jian Jeffrey, Santa Clara, CA, United States  
Hendricks, Robert Nam, Palo Alto, CA, United States  
Melnick, Michael Joseph, San Diego, CA, United States  
Walker, Keith Adam Murray, Los Altos Hills, CA, United States  
PA Syntex (US) Inc., Palo Alto, CA, United States (U.S. corporation)  
Apurion Pharmaceuticals, Inc., San Diego, CA, United States (U.S. corporation)  
P1 US 8130220 20001010  
AI US 1999368677 19990805 (9)  
RU Division of Ser. No. US 199839651, filed on 21 Jan 1998  
PRA1 US 199836714 19980714  
US 199767209 19970116 (60)

LREP Peries, Rohan, Bansal, Rekha  
 CLMN Number of Claims: 44  
 ECL Exemplary Claim: 1  
 DRAWN No Drawings  
 LN CNT 5004  
 CASINDEXING IS AVAILABLE FOR THIS PATENT  
 AB This invention relates to sulfonamides of formula (I) ##STR1## that are  
 inhibitors of metalloproteases, pharmaceutical compositions containing  
 them, methods for their use and methods for preparing these compounds

U5 ANSWER 9 OF 17 USPATFULL  
AN 2000 97998 USPATFULL  
T1 Agent for suppressing a reduction of CD4 sup. + lymphocytes  
IN Nakamura, Norio, Tokyo, Japan  
Shirakawa, Kamon, Tokyo, Japan  
Matsuse, Tomokazu, Tokyo, Japan  
Nagata, Shingekazu, Suita, Japan  
Co., Man, Sung, Cupertino, CA, United States  
Vasquez, Maximiliano, Palo Alto, CA, United States  
PA Mochida Pharmaceutical Co., Ltd., Tokyo, Japan (non-U.S. corporation)  
Osaka Bioscience Institute, Osaka-Fu, Japan (non-U.S. corporation)  
PI US 6066312, 200009801  
RI US 1998-9999631, 19980122 (B)  
A1 Continuation-in-part of Ser. No. US 1987-1011, filed on 30 Dec 1987, now  
abandoned which is a continuation-in-part of Ser. No. WO/1996-01P1820,  
filed on 1 Jul 1995 which is a continuation-in-part of Ser. No. US  
1996-649100, filed on 17 May 1996  
PRA1 JP 1995-168480 19950630

DT Utility  
EXNAM Primary Examiner Kemmerer, Elizabeth Assistant Examiner Basu, Nirmla  
S  
CLM# Number of Claims 4  
ECL Exemplary Claim 1  
DRAWN No Drawings  
LNCNT 615  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB An agent for preventing or treating AIDS which contains as its effective component an anti-Fas ligand antibody and the method for preventing and treating AIDS by using such drug

L5 ANSWER 10 OF 17 USPATFULL  
AN 2000 7385 USPATFULL  
T1 Soluble divalent and multivalent heterodimeric analogs of proteins  
IN Schreck, Jonathan Silver Spring, MD, United States  
O'Brien, Sean, Baltimore, MD, United States  
PA The Johns Hopkins University, Baltimore, MD, United States (U S  
corporation)  
PI US 6015684 20000118  
AI US 1997-828712 19970328 (6)  
PRI1 US 1996-14367 19960328 (60)  
DT Utility  
EXNAM1 Primary Examiner Hutzell, Paula K., Assistant Examiner Bansal,  
Geetha

CLAIM: Number of Claims: 10  
ECL: Exemplary Claim: 1  
DRAW: 18 Drawing Figure(s), 16 Drawing Page(s)  
LN CN: LN CN 2027  
CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB: Specifically in immune responses is in part controlled by the selective interaction of T cell receptors with their cognate ligands, peptide/MHC molecules. The discriminating nature of this interaction makes these molecules, in soluble form, good candidates for selectively regulating immune responses. Attempts to exploit soluble analogs of these proteins has been hampered by the intrinsic low avidity of these molecules for their ligands. To increase the avidity of soluble analogs for their cognates, to biologically relevant levels, divalent peptide/MHC complexes or T cell receptors (superdimers) were constructed. Using a recombinant DNA strategy, DNA encoding either the MHC class II peptide or TCR heterodimers was ligated to DNA coding for murine Ig heavy and light chains. These constructs were subsequently expressed in a baculovirus expression system. Enzyme-linked immunosorbent assays (ELISA) specific for the Ig and polymorphic determinants of either the TCR or MHC fraction of the molecule indicated that infected insect cells secreted approximately 1 mu g/ml of soluble conformationally intact chimeric superdimers. SDS-PAGE gel analysis of purified protein showed that expected molecular weight species. The results of flow cytometry demonstrated that the TCR and class II chimeras bound specifically with high avidity to cells bearing their cognate receptors. These superdimers will be useful for studying TCR/MHC interactions, lymphocyte tracking, identifying new antigens, and have possible uses as specific regulators of immune responses.

L5 ANSWER 11 OF 17 USPTA FULL  
AN 2000 7061 USPTA FULL  
T1 Gas abductors  
IN Lynch, David H., Bainbridge Island, WA, United States  
PA Alderson, Mark R., Bainbridge Island, WA, United States  
P1 Immature Corporation, Seattle, WA, United States (U S corporation)  
AI US 6015559 20000118  
RI US 1988-152733 19890801 (S)  
Pat. No. US 5630469 which is a continuation-in-part of Ser. No. US 1993-422805, filed on 13 Oct 1994, now patented  
Pat. No. US 5630469 which is a continuation-in-part of Ser. No. US 1994-322805, filed on 13 Oct 1994, now patented  
Pat. No. US 5630469 which is a continuation-in-part of Ser. No. US 1993-159003, filed on 29 Nov 1993, now abandoned  
1993-159617, filed on 14 Oct 1993, now abandoned

DI Utility  
EXNAM Primary Examiner: Chin, Christopher L., Assistant Examiner: Dem, S  
LREP Anderson, Kathryn A  
CLMN Number of Claims 17  
ECL Exemplary Claim 1  
DRAWN 14 Drawing Figure(s) 10 Drawing Page(s)

IN CNT 2134

CAS INDEXING IS AVAILABLE FOR THIS PATENT

AB The present invention provides a panel of monoclonal antibodies and binding proteins which specifically bind to human Fas antigen. Some of the antibodies and binding proteins are capable of stimulating T cell proliferation, inhibiting binding of anti-Fas CH-11 monoclonal antibody to cells expressing Fas antigen, blocking anti-Fas CH-11 monoclonal antibody-mediated lysis of cells, and blocking Fas ligand-mediated lysis of cells. The invention also provides for therapeutic compositions comprising the monoclonal antibodies.

US ANSWER 12 OF 17 USPATFULL  
AN 1999 160028 USPATFULL  
TI Sulfamide metalloprotease inhibitors  
IN Brock, Chris Allen, Foster City, CA, United States  
Campbell, Jeffrey Allen, Fremont, CA, United States  
Castellano, Alindo Lucas, New City, NY, United States  
Chen, Jian Jeffrey, Santa Clara, CA, United States  
Hendricks, Robert Than, Palo Alto, CA, United States  
Meinick, Michael Joseph, San Diego, CA, United States  
Walker, Keith Adrian Murray, Los Altos Hills, CA, United States  
Syntex (U S A) Inc., Palo Alto, CA, United States (U S corporation)  
Agouron Pharmaceuticals, Inc., San Diego, CA, United States (U S corporation)  
P1 US 5998412, 19991207  
A1 US 1998 9951, 19990121 (9)  
PRA1 US 1997 36714, 19970123 (60)  
US 1997 62209 19971016 (60)  
DI Utility  
EXNAM Primary Examiner: Raymond, Richard L  
LREP Perles, Rohan, Bernal, Rekha  
CLMN Number of Claims 15  
ECL Exemplary Claim 1  
DRWN No Drawings  
LN CNT 4881

CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB This invention relates to sulfamides of formula (I) ##STR1## that are inhibitors of metalloproteases, pharmaceutical compositions containing them, methods for their use and methods for preparing these compounds

US ANSWER 13 OF 17 USPATFULL

AN 1999 89171 USPATFULL

TI Matrix metalloprotease inhibitors  
IN Bender, Steven Lee, Oceanside, CA, United States  
Brock, Chris Allen, Foster City, CA, United States  
Campbell, Jeffrey Allen, Fremont, CA, United States  
Castellano, Alindo Lucas, New York, NY, United States  
Fisher, Lawrence Emerson, Mountain View, CA, United States  
Hendricks, Robert Than, Palo Alto, CA, United States  
Sarna, Keshab, Sunnyvale, CA, United States  
Syntex (U S A) Inc., Palo Alto, CA, United States (U S corporation)  
Agouron Pharmaceuticals, Inc., San Diego, CA, United States (U S corporation)  
P1 US 5932595, 19990803  
A1 US 1996 76904, 19961218 (6)  
PRA1 US 1996 22439 19961220 (60)  
US 1996 8939 19961204 (60)  
US 1996 32096 19961204 (60)  
DI Utility  
EXNAM Primary Examiner: Kight, John, Assistant Examiner: Covington, Raymond  
LREP Perles, Rohan  
CLMN Number of Claims 60  
ECL Exemplary Claim 1  
DRWN No Drawings  
LN CNT 4966

CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB The present invention relates to compounds of formula I ##STR1## that are matrix metalloprotease inhibitors, pharmaceutical compositions containing them, methods for their use and methods of preparing these compounds

US ANSWER 14 OF 17 USPATFULL  
AN 1999 4647 USPATFULL

TI Fas ligand compositions for treatment of proliferative disorders

IN Wash, Kenneth, Carle, MA, United States  
PA St Elizabeth's Medical Center, Boston, MA, United States (U S corporation)  
P1 US 5858990, 19990112  
A1 US 1997 810453, 19970304 (8)  
DI Utility  
EXNAM Primary Examiner: Elliott, George C., Assistant Examiner: McGarry, Sean  
LREP Wolf, Greenfield & Sachs, P C  
CLMN Number of Claims 9  
ECL Exemplary Claim 1  
DRWN No Drawings  
LN CNT 3038

CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB A method for treating vascular injury, particularly vascular injury resulting from restenosis following angioplasty and vascular remodeling is provided. The method involves administering to subjects in need of such treatment an effective amount of a Fas ligand molecule

US ANSWER 15 OF 17 USPATFULL

AN 1998 134631 USPATFULL  
TI Fas antagonists and uses thereof  
IN Lynch, David H., Barnbridge Island, WA, United States  
Alderson, Mark R., Barnbridge Island, WA, United States  
Immuner Corporation, Seattle, WA, United States (U S corporation)  
P1 US 5830469, 19981103  
A1 US 1995 42949, 19950426 (8)  
R1 Continuation-in-part of Ser. No. US 1994 322805, filed on 13 Oct 1994  
R2 Continuation-in-part of Ser. No. US 5620889 which is a continuation-in-part of Ser. No. US 1993 158003, filed on 28 Nov 1993, now abandoned which is a continuation-in-part of Ser. No. US 1993 136817, filed on 14 Oct 1993, now abandoned  
DI Utility  
EXNAM Primary Examiner: Long, Susan A  
LREP Anderson, Kathryn A  
CLMN Number of Claims 26  
ECL Exemplary Claim 1  
DRWN 14 Drawing Figure(s), 10 Drawing Page(s)  
LN CNT 1997

CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB The present invention provides a panel of monoclonal antibodies and binding proteins which specifically bind to human Fas antigen. Some of the antibodies and binding proteins are capable of stimulating T cell proliferation, inhibiting binding of anti-Fas CH-11 monoclonal antibody to cells expressing Fas antigen, blocking anti-Fas CH-11 monoclonal antibody-mediated lysis of cells, and blocking Fas ligand-mediated lysis of cells. The invention also provides for therapeutic compositions comprising the monoclonal antibodies

US ANSWER 16 OF 17 USPATFULL

AN 1998 134627 USPATFULL  
TI Yeast-based delivery vehicles  
IN Duke, Richard C., Denver, CO, United States  
Franzuso, Alex, Boulder, CO, United States  
Beligau, Donald, Denver, CO, United States  
University Technology Corporation, Boulder, CO, United States (U S corporation)  
P1 US 5830463, 19981103  
A1 US 1994 340185, 19941115 (8)  
R1 Continuation-in-part of Ser. No. US 1993 86322, filed on 7 Jul 1993, now patented, Pat. No. US 5413614  
DI Utility  
EXNAM Primary Examiner: Chambers, Jasmine C., Assistant Examiner: Hauck, Karen M  
LREP Sheridan Ross P C  
CLMN Number of Claims 12  
ECL Exemplary Claim 1, 12  
DRWN 3 Drawing Figure(s), 3 Drawing Page(s)  
LN CNT 1929

CAS INDEXING IS AVAILABLE FOR THIS PATENT

AB The present invention includes yeast vehicles and their use as delivery vehicles. Yeast vehicles include a yeast portion and a heterologous compound. Such yeast vehicles can be used to protect animals from disease and to otherwise carry compounds to given cell types. Examples of yeast vehicles include gene delivery vehicles, drug delivery vehicles, and immunomodulatory vehicles. Immunomodulatory vehicles are capable of modulating an immune response. When stimulating an immune response, such yeast vehicles effect cell-mediated as well as humoral immunity

US ANSWER 17 OF 17 USPATFULL

AN 1998 61196 USPATFULL  
TI Use of Fas ligand to suppress T lymphocyte mediated immune responses  
IN Beligau, Donald, Denver, CO, United States  
Duke, Richard C., Denver, CO, United States  
University Technology Corporation, Boulder, CO, United States (U S corporation)  
P1 US 5759536, 19980602  
A1 US 1995 378507, 19950126 (8)  
R1 Continuation-in-part of Ser. No. US 1994 250478, filed on 27 May 1994  
DI Utility  
EXNAM Primary Examiner: Campbell, Bruce R  
LREP Sheridan & Ross, P C  
CLMN Number of Claims 7  
ECL Exemplary Claim 1  
DRWN No Drawings  
LN CNT 802

CAS INDEXING IS AVAILABLE FOR THIS PATENT  
AB A method for inhibiting T lymphocyte-mediated immune responses, including those directed against autologous and/or heterologous tissues, e.g. by a recipient mammal of a transplanted tissue, said method comprising providing the recipient mammal with Fas ligand. The Fas ligand may be provided to the recipient mammal by a variety of means, including by pump implantation or by transplantation of transgenic tissue expressing Fas ligand. Also provided is a method for diagnostic use of Fas ligand expression in improving transplantation success

=> d his

(FILE HOME ENTERED AT 10 02 46 ON 25 MAY 2001)

FILE MEDLINE, BIOSIS, EMBASE, CANCERLIT, SCISEARCH ENTERED AT 10 03 22  
ON 25 MAY 2001  
L1 11361 S FAS LIGAND  
L2 3148 S L1 AND (ANTIBOD? OR MONOCLON?)  
L3 88 S L2 AND (GRAFT VERSUS HOST OR GVHD)  
L4 37 DUP REM 13 (51 DUPLICATES REMOVED)

FILE USPATFULL ENTERED AT 10 13 02 ON 25 MAY 2001  
L5 17 S L2 AND (GRAFT VERSUS HOST OR GVHD)

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# Induction of Fas (Apo-1, CD95)-Mediated Apoptosis of Activated Lymphocytes by Polyclonal Antithymocyte Globulins

By Laurent Genestier, Sylvie Fournel, Monique Flacher, Olga Assossou, Jean-Pierre Revillard, and Nathalie Bonnefoy-Berard

Polyclonal horse antilymphocyte and rabbit antithymocyte globulins (ATGs) are currently used in severe aplastic anemia and for the treatment of organ allograft acute rejection and graft-versus-host disease. ATG treatment induces a major depletion of peripheral blood lymphocytes, which contributes to its overall immunosuppressive effects. Several mechanisms that may account for lymphocyte lysis were investigated *in vitro*. At high concentrations (.1 to 1 mg/mL) ATGs activate the human classic complement pathway and induce lysis of both resting and phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells. At low, submitogenic, concentration ATGs induce antibody-dependent cell cytotoxicity of PHA-activated cells, but not resting cells.

THE POLYCLONAL antilymphocyte or antithymocyte globulins (ATG)\* are potent immunosuppressive agents used in organ transplantation since the late 1960s. They have proved effective either as rescue treatment of first rejection episodes and graft-versus-host reaction or as prophylactic treatment of rejection.<sup>1</sup> As an alternative to polyclonal ATGs, monoclonal antibody (MoAb) OKT3 has been extensively used in organ transplantation.<sup>2,3</sup> However, in clinical studies, polyclonal ATGs compare favorably to OKT3 both for prophylactic use or in rescue therapy.<sup>4</sup> The precise mechanism of action of ATGs is undefined, but the profound lymphocytopenia observed throughout the treatment period mainly contributes to the immunosuppressive effect. Various mechanisms have been proposed to explain lymphocyte depletion, including complement-mediated cytolysis or clearance of lymphocytes by opsonization and phagocytosis by macrophages.<sup>5</sup> ATGs are a mixture of multiple antibodies to various lymphocyte surface antigens.<sup>6-8</sup> It was recently reported that antibodies specific for HLA class I molecules,<sup>9-11</sup> and antibodies to CD2,<sup>12-13</sup> CD30,<sup>14</sup> CD45,<sup>15</sup> and CTLA-4<sup>16</sup> could induce apoptosis of T cells, whereas anti-HLA class II and anti-HLA class I antibodies can also trigger apoptosis of activated B cells.<sup>17</sup> Antibodies to CD2, CD3, CD45, and HLA molecules were identified in ATGs; it may therefore be hypothesized that their binding either to

They also trigger surface Fas (Apo-1, CD95) expression in naive T cells and Fas-ligand gene and protein expression in both naive and primed T cells, resulting in Fas/Fas-L interaction-mediated cell death. ATG-induced apoptosis and Fas-L expression were not observed with an ATG preparation lacking CD2 and CD3 antibodies. Susceptibility to ATG-induced apoptosis was restricted to activated cells, dependent on IL-2, and prevented by Cyclosporin A, FK506, and rapamycin. The data suggest that low doses of ATGs could be clinically evaluated in treatments aiming at the selective deletion of *in vivo* activated T cells in order to avoid massive lymphocyte depletion and subsequent immunodeficiency.  
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resting or to activated T cells, or both, may trigger a signal of programmed cell death. Furthermore, ATGs contain antibodies to CD2 and CD3, which account for their mitogenic properties.<sup>7</sup> Repeated activation of mature T cells through CD2 or CD3 results in apoptosis of activated T cells.<sup>18</sup> The major pathway of this activation-induced cell death (AICD) uses the interaction between Fas (Apo-1, CD95) expressed by activated T and B cells and Fas-ligand (Fas-L, CD95-L) produced by a subset of activated T cells.<sup>19-21</sup> The present study was designed to investigate *in vitro* the different mechanisms whereby ATGs can induce peripheral lymphocyte depletion. To this end, we measured the capacity of ATGs bound to peripheral blood lymphocytes (PBL) to bind human C1q and to induce complement-dependent lysis. We determined their activity in antibody-dependent cell-mediated cytotoxicity (ADCC) and their capacity to induce Fas and Fas-L expression. In all those assays, we compared the sensitivity of naive versus mitogen-activated PBL to ATG-induced lysis, in order to identify those mechanisms that could display some specificity toward preactivated PBL. The dose responses were analyzed according to serum concentrations achieved during treatments. Finally, we evaluated the effect of immunosuppressive drugs that interfere with the interleukin-2 (IL-2) pathway (Cyclosporin A, [CsA], FK506, rapamycin) on the development of the sensitivity to ATG-induced lysis.

\*Within the context of this report, ATG is used to refer to either antithymocyte or antilymphocyte globulins.

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The first two authors contributed equally to this work and therefore share the first authorship.

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## MATERIALS AND METHODS

**Antibodies and reagents.** Rabbit ATG, batch no. 95-07, and horse antilymphocyte globulins, batches no. 1141 and no. 5, were provided by Dr J. Carcagne (Pasteur Merieux serums & vaccins, Lyon, France). Characteristics of each batch have been previously reported.<sup>7</sup> F(ab')<sub>2</sub> fragments of ATG no. 95-07 were prepared by pepsin digestion and purified by exclusion chromatography on protein A, following standard procedures. Normal rabbit IgG (Zymed, San Francisco, CA) and horse anti-rabies globulins purified according to the same procedure used for ATGs (Pasteur Merieux serums & vaccins) were used as controls. The anti-CD52 MoAb CAMPATH-1M (IgM) was a gift from Prof H. Waldmann (Sir Dunn School of Pathology, University of Oxford, Oxford, UK). The three anti-Fas MoAbs were used in this study, UB2 for cytofluorometry assays; CH11 (IgM), ZB4 (IgG1), and phycoerythrin streptavidin were obtained from Immunotech (Marseille, France).

Fluorescein-isothiocyanate (FITC)-conjugated CD25 and CD69 MoAbs were obtained from Becton Dickinson (Mountain View, CA) and two biotinylated anti-Fas-L one from Pharmingen (San Diego, CA) and the other from Alexis Corporation (Cogen S.A., Paris, France). CD3 MoAb OKT3 was from Cilag Laboratories (Levallois-Perret, France).

The lectin phytohemagglutinin (PHA), phorbol myristate acetate (PMA), ionomycin, and cycloheximide (CHX) were obtained from Sigma Chemical Co. (St. Louis, MO). Rapamycin (RPM) and FK506 were gifts from Dr A. Altman (La Jolla Institute for Allergy and Immunology, San Diego, CA), and CsA was kindly supplied by Sandoz (Novartis, Paris, France). Human IL-2 and rIFN- $\gamma$  were kindly provided by Dr J. Banchemereau (Schering-Plough, Dardilly, France).

**Cell preparation.** Peripheral blood was collected from healthy donors in the presence of sodium citrate. After the addition of a calcium chloride solution, blood was defibrinated by gentle rotation of the flask; mononuclear cells were then isolated by centrifugation on a layer of Histopaque (Sigma). Cells were washed three times in Hank's balanced salt solution (HBSS) before culture. Those cell suspensions referred to as PBL were shown to contain  $3.8\% \pm 0.4\%$  monocytes, as defined by expression of CD14. For complement-mediated lysis and ADCC experiments, peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of heparinized blood on a layer of Histopaque.

**Culture medium and cell proliferation.** PBL were resuspended in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (FCS), 2 mmol/L L-glutamine, and antibiotics (penicillin 100 U/mL, streptomycin 100  $\mu$ g/mL). For the proliferation assay, cells ( $10^6$ /mL) were incubated in 96-well microplates (Costar, Cambridge, MA) in the presence of PHA (5  $\mu$ g/mL) or with ATGs at the indicated concentrations. Cultures were maintained in a humid atmosphere at 37°C containing 5% CO<sub>2</sub> for the indicated time.

**Immunofluorescence assays.** Cells were washed with isotonic NaCl/Pi buffer containing 1% bovine serum albumin (BSA) and 0.2% NaN<sub>3</sub> (phosphate-buffered saline [PBS]/BSA/azide). Cells ( $5 \times 10^5$ ) were incubated with 10  $\mu$ L labeled MoAbs for 30 minutes at 4°C. Then, after two washes in PBS/BSA/azide buffer, cells were fixed with 1% formaldehyde in PBS/BSA/azide buffer and analyzed by flow cytometry with a FACScan (Becton Dickinson, Pont de Claix, France). For intracellular analysis of Fas-L expression, cells were fixed with freshly prepared 2% paraformaldehyde in PBS and permeabilized by saponin (0.33%) (Sigma).

**Measurement of apoptosis.** After 3 days of culture, unstimulated or PHA-activated PBL were harvested. Dead cells were removed by centrifugation on a layer of Histopaque (Sigma), and viable cells were washed in HBSS. Viable cells ( $10^6$ /mL) were incubated in 96-well microplates in the presence of ATG or CH11 MoAb. After incubation, cell death was evaluated by three different techniques. Measurement of mitochondrial transmembrane potential by flow cytometry after 3,3'-dihexyloxacarbocyanine (DiOC<sub>6</sub>) staining<sup>22</sup> and detection of phosphatidylserine expression by flow cytometry after addition of FITC-conjugated annexin V<sup>23</sup> were performed on the same suspensions at the indicated time. Nuclear apoptosis was assessed by fluorescence microscopy after staining with Hoechst 33342 (Sigma) at 10  $\mu$ g/mL, following previously described methods.<sup>23</sup> Nuclear fragmentation or marked condensation of the chromatin with reduction of nuclear size, or both, were considered typical features of apoptotic cells. On the basis of these measurements, results were expressed either as percentage of apoptotic cells or as percentage of specific apoptosis according to the formula

$$\% \text{ Specific Apoptosis} = \frac{(\text{test} - \text{control}) \times 100}{(100 - \text{control})}$$

**RNA isolation, reverse transcription, PCR amplification of Fas-L and mRNA, and quantification.** Total cellular RNA was isolated from  $5 \times 10^6$  cells, following the method of Chomczynski and Sacchi.<sup>25</sup> Reverse transcription of 1  $\mu$ g RNA was performed using the first-stand

cDNA synthesis kit (Pharmacia Biotech, Orsay, France) in a total reaction volume of 15  $\mu$ L. After 90 minutes at 37°C, the reaction was terminated by heating for 4 minutes at 95°C. PCR was performed in mixtures containing 1  $\mu$ L cDNA derived from 10 ng total RNA, primers (100 ng of each; Eurogentech, Seraing, Belgium), 2.5  $\mu$ L 10 $\times$  PCR buffer (Promega, Charbonnières, France) containing 1.5 mmol/L MgCl<sub>2</sub>, 0.05 mmol/L of each dNTP, and 0.5 U of Taq polymerase (Promega). Primers for Fas-L and Actin included Fas-L sense primer 5'-CCA-TTT-AAC-AGG-CAA-GTC-CAA-CTC-3', Fas-L anti-sense primer 5'-CAA-CAT-TCT-CGG-TGC-CTG-TAA-C-3', actin sense primer 5'-GGG-TCA-GAA-GGA-TTC-CTA-TG-3', and actin anti-sense primer 5'-GGTCTCAAACATGSACTCTGGG-3'. These primers were designed to discriminate between the amplification of cDNA (low size PCR products) and contaminating genomic cDNA (high size PCR products). For each amplicon, 23 to 35 amplification cycles (1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C) were performed with the PCR system 9600 (Perkin Elmer, Montigny-le Bretonneux, France). Semi-quantitative evaluation of amplification products was performed as described by Morgan et al.<sup>26</sup> Briefly, each PCR product (15  $\mu$ L) was electrophoresed on agarose gel (2%) stained with ethidium bromide and photographed using polaroid type 665 positive/negative film. The specificity of PCR reaction was confirmed by the expected size of the amplification products. The PCR signal intensities were quantitated by scanning the negative film using a Desktop Scanning Densitometer (PDI/Pharmacia Biotech, Saint-Quentin-Yvelines, France) and by evaluating the integrated trace optical density (OD) for each band using Quantity One Software (PDI/Pharmacia Biotech). The point for samples comparison in the exponential amplification range was selected by inspection from semi-logarithmic plots of OD versus cycle numbers. To correct for variations in the amount of input cDNA, results were expressed as the ratio Fas-L OD/actin OD at the point previously determined.

**Complement-mediated lysis.** Resting or PHA-activated PBMC were labeled with Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub> for 2 hours at room temperature and washed twice. They were resuspended in medium at  $2 \times 10^6$  cells/mL, and 100  $\mu$ L of the suspension was added to round-bottomed microtiter plates containing 50  $\mu$ L of an appropriate dilution of the antibody. After incubation for 10 minutes at room temperature, 50  $\mu$ L of 40% fresh or heat-inactivated (56°C, 30 minutes) autologous serum (obtained from defibrinated blood) was added. The cell suspensions were incubated at 37°C for 30 minutes, then centrifuged at 100g for 2 minutes, and 100  $\mu$ L of the supernatant was collected for measurement of released radioactivity. Controls without antibody were used to measure the spontaneous radioactivity release. The percentage of specific <sup>51</sup>Cr release was calculated using the formula

$$\text{Specific Release} = \frac{(\text{test} - \text{spontaneous}) \times 100}{(\text{total} - \text{spontaneous})}$$

**C1q binding.** A total of 20  $\mu$ L of ATGs or control Ig in PBS/BSA/azide was added to PBMC pellets ( $4 \times 10^5$ ) and incubated at 37°C for 30 minutes. After two washes in PBS, samples were separated in two and incubated at room temperature for 30 minutes in the presence of 50  $\mu$ L of autologous serum or heat-inactivated (56°C, 30 minutes) serum as a control. After two washes, cells were incubated with 10  $\mu$ L of polyclonal goat anti-C1q FITC antibody (1/50 Cappel, Durham, NC) at 4°C for 30 minutes. After two washes, cells were fixed with 1% formaldehyde in PBS/BSA/azide buffer and analysis performed on a FACScan flow cytometer.

**Antibody-dependent cell cytotoxicity.** Resting and PHA-activated PBMC were labeled with Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub> for 2 hours at room temperature and washed twice. They were resuspended in medium at  $1 \times 10^6$  cells/mL, and 50  $\mu$ L of the suspension was added to round-bottomed microtiter plates containing 50  $\mu$ L of an appropriate dilution of the antibody. After incubation for 10 minutes at room temperature, 100  $\mu$ L

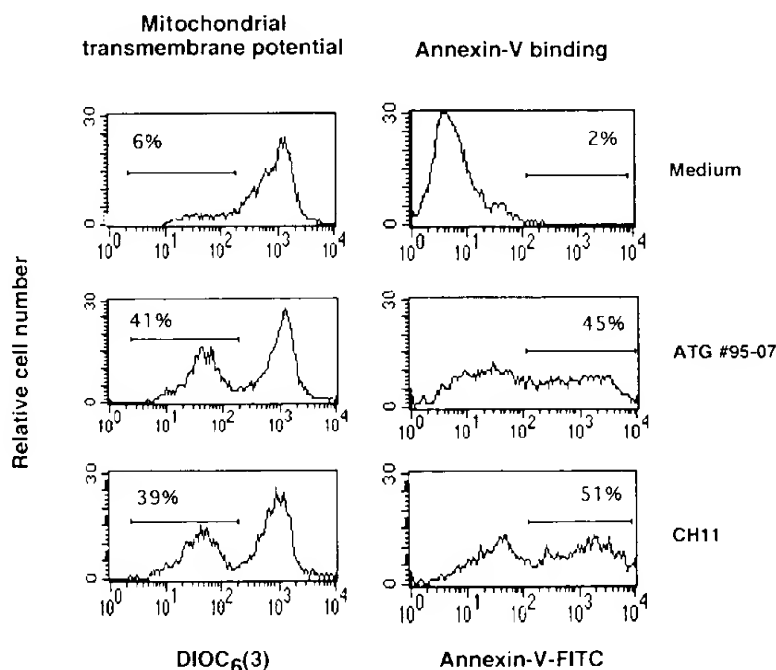
of effector cells ( $25 \times 10^6$  cells/mL) was added. The cell suspensions were incubated at 37°C for 6 hours, then centrifuged at 100g for 2 minutes and 100  $\mu$ L of the supernatant collected for measurement of released radioactivity as for complement-mediated lysis.

## RESULTS

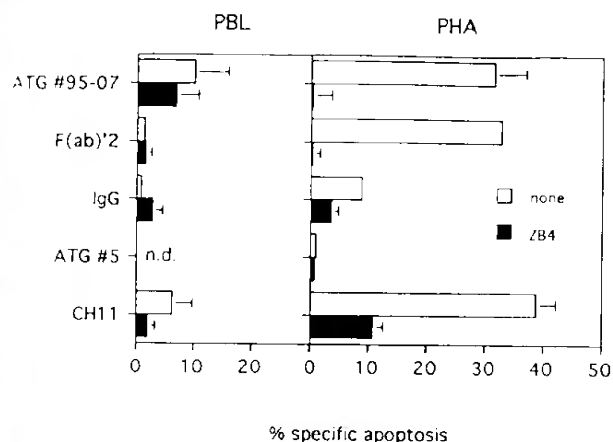
**ATGs induce apoptosis of activated lymphoblasts.** Knowing that ATGs could induce apoptosis of B-cell lines and to a lesser extent, T-cell lines,<sup>27</sup> we examined whether such mechanism could also take part in the elimination of peripheral T lymphocytes. Three-day PHA-activated PBL, as well as nonactivated PBL, were treated with ATG no. 95-07, F(ab')<sub>2</sub> fragments of ATG, anti-Fas MoAb CH11 as positive control, and normal rabbit IgG as negative control. Apoptosis was evaluated by DiOC<sub>6</sub>(3) and annexin V staining (Fig 1) and by fluorescence microscopy after staining with Hoechst 33342 (Fig 2). The results showed that ATG no. 95-07 at nonmitogenic concentrations (10  $\mu$ g/mL), their F(ab')<sub>2</sub> fragments, and the anti-Fas MoAb CH11 induced apoptosis of 30% to 40% of PHA-activated PBL, whereas resting PBL were not sensitive (Figs 1 and 2). Similar results were observed with ATG no. 1141 obtained from horse (data not shown). Interestingly ATG no. 5 containing CD18, CD11a, anti- $\beta$ 2m, and anti-HLA DR antibodies, but no CD3, CD2, and CD5 specificities, and which is not mitogenic at concentrations ranging from 1 to 1,000  $\mu$ g/mL, did not induce apoptosis at 10 and 100  $\mu$ g/mL (Fig 2; data not shown). Normal rabbit did not induce cell death of resting or activated PBL (Fig 2). Similar experiments were repeated with PBL activated by a 3-day culture period with PMA (10 ng/mL) plus ionomycin (500 ng/mL), PMA (10 ng/mL) plus OKT3 (100 ng/mL), or a mitogenic concentration of ATG no. 95-07 or no. 1141 (100  $\mu$ g/mL). Whatever the activator used, the addition of ATGs (10  $\mu$ g/mL) or F(ab')<sub>2</sub> fragments thereof resulted in specific apoptosis ranging from 20% to 50% (data not shown).

**ATG-induced apoptosis is fully inhibited by an antagonist anti-Fas antibody.** The apoptotic activity of ATGs was effective only on activated T cells, which express Fas and which are sensitive to Fas-mediated apoptosis<sup>28</sup>; we therefore studied whether ATG-induced apoptosis was dependent on Fas/Fas-L interaction. To this end, PHA-activated PBL were incubated for 1 hour with the antagonist anti-Fas MoAb ZB4, which blocks the interaction between Fas and Fas-L, before addition of ATG no. 95-07, ATG F(ab')<sub>2</sub> fragments or CH11 MoAb. As shown in Fig 2, ATG-induced apoptosis was completely blocked by ZB4, indicating that ATG-induced apoptosis of activated T cells required Fas/Fas-L interaction. This idea was re-enforced by the observation that simultaneous addition of ATG no. 95-07 (10  $\mu$ g/mL) and CH11 resulted in the same percentage of apoptotic cells as with each antibody tested alone (data not shown). This result suggests that the same subset of activated T cells is the target of ATGs and anti-Fas antibodies. Furthermore, it shows that ATGs do not contain anti-Fas blocking antibodies, at least in sufficient amount to be detected in this assay.

**ATGs induce Fas and Fas-L expression.** In an effort to obtain further evidence for a possible role of Fas/Fas-L interaction in ATG-induced apoptosis, we examined whether ATGs would induce Fas-L expression in both resting and activated-PBL. To this end, PBL were first cultured in presence of a mitogenic concentration of ATG no. 95-07 (100  $\mu$ g/mL) or PHA or medium alone for 3 days. After elimination of dead cells, preactivated PBL were then incubated for 6 hours with medium alone, ATG no. 95-07 at nonmitogenic (10  $\mu$ g/mL) and mitogenic (100  $\mu$ g/mL) concentrations or PHA, and induction of Fas-L mRNA was analyzed by RT-PCR. ATG no. 95-07 at either 10 or 100  $\mu$ g/mL induced Fas-L mRNA expression by nonactivated and by preactivated-PBL (Fig 3). Similar experiments performed with freshly isolated PBL showed that ATG no.



**Fig 1.** Effect of ATGs on mitochondrial transmembrane potential and on phosphatidylserine expression. PBL were activated for 3 days in presence of PHA (5  $\mu$ g/mL). After removal of dead cells, medium alone, ATG no. 95-07 (10  $\mu$ g/mL), or CH11 anti-Fas MoAb (1  $\mu$ g/mL) was added. After 12 hours,  $\Delta\Psi_m$  modifications were evaluated by staining with DiOC<sub>6</sub>(3). The expression of phosphatidylserine at the surface membrane was evaluated after 15 hours by measuring annexin-V binding. The percentage of cells with decreased mitochondrial potential membrane or increased expression of phosphatidylserine are indicated for each histogram. Results from one typical experiment among four showing similar percentages.



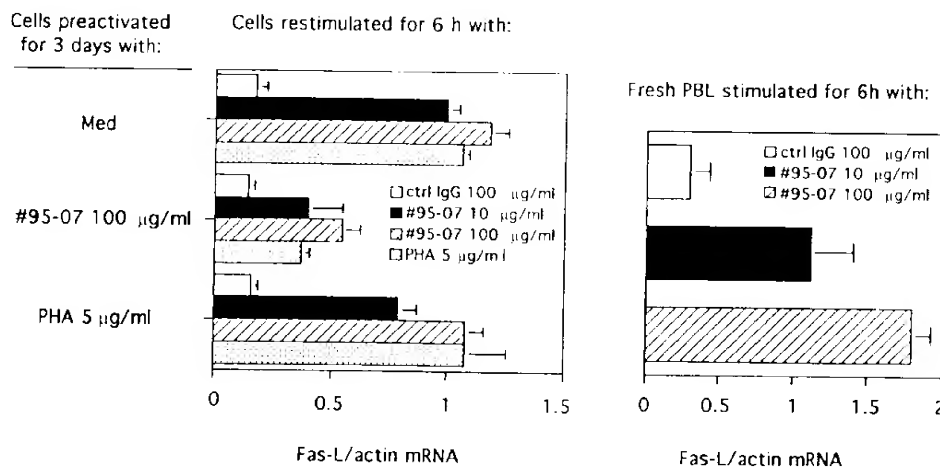
**Fig 2. ATGs induce apoptosis of activated T lymphocytes.** PBL were cultured in presence of medium alone or PHA (5  $\mu\text{g/mL}$ ) for 3 days. Dead cells were removed and viable cells were treated for 20 hours with ATG no. 95-07,  $\text{F(ab')}_2$  fragments of ATG no. 95-07, ATG no. 5 or normal rabbit IgG at 10  $\mu\text{g/mL}$  or with the agonist anti-Fas MoAb CH11 at 1  $\mu\text{g/mL}$ . Protection by the antagonist anti-Fas MoAb, was tested by pre-incubating PBL or PHA-activated cells for 1 hour with ZB4 MoAb at 2  $\mu\text{g/mL}$ . The percentage of apoptotic cells was determined by fluorescent microscopy after staining with Hoechst 33342. Results are expressed as mean  $\pm$  SEM of five different experiments or as mean of two experiments for ATG no. 5.

95-07 (10 and 100  $\mu\text{g/mL}$ ), but not control rabbit IgG, strongly induced Fas-L mRNA expression (Fig 3).

In parallel, surface expression of Fas and Fas-L molecules, but CD25 and CD69 activation markers as well, was analyzed by flow cytometry on PBL cultured in the presence of ATG no. 95-07 at 10 and 100  $\mu\text{g/mL}$  for 1 to 3 days. At mitogenic concentrations (100  $\mu\text{g/mL}$ ), ATG no. 95-07 induced CD69, CD25, Fas, and Fas-L expression (Fig 4). Surface expression of Fas, CD69, and CD25 reached a maximum at day 2, and that of

Fas-L at day 1. At nonmitogenic concentrations (ie, 10  $\mu\text{g/mL}$ ), ATG no. 95-07 still induced expression of CD69, Fas, and Fas-L, but not that of the CD25 molecule, suggesting that, at low concentrations, ATGs drive lymphocytes into the  $G_1$  phase of the cell cycle but did not allow them to progress to S phase because of the absence of CD25 expression. Interestingly ATG no. 5 at 100  $\mu\text{g/mL}$  did not induce CD69 Fas and Fas-L expression (Fig 4), nor did it trigger apoptosis (Fig 2). Finally, these experiments were completed by intracellular staining of Fas-L in paraformaldehyde-fixed and saponin-permeabilized cells. The results indicate that ATG no. 95-07 (10 and 100  $\mu\text{g/mL}$ ) increased intracellular Fas-L in both resting and preactivated PBL, with a maximum on days 1 to 2 (Fig 4; data not shown). Histograms of fluorescence (Fig 4) show that a small subset of PBL is positive before activation, whereas after stimulation by ATG, most of the lymphocyte population becomes Fas-L positive.

*Interference with the IL-2 pathway reduces ATGs-induced apoptosis.* Knowing that IL-2 is required for acquisition of susceptibility to Fas-mediated apoptosis,<sup>29,30</sup> we analyzed the effect of immunosuppressive agents that interfere with the IL-2 pathway on ATG-induced cell death. PBL were cultured with PHA in the presence of CsA or FK506, which block IL-2 expression at a transcriptional level, or with RPM, which blocks IL-2 signaling. After 3 days, cells were treated with ATGs or  $\text{F(ab')}_2$  fragments. The presence of CsA, FK506, or RPM, during T-cell activation, markedly decreased apoptosis mediated by ATG no. 95-07 or their  $\text{F(ab')}_2$  fragments (Fig 5). In keeping with these results, we observed that addition of rIL-2 during the last 24 hours of cell culture, to PBL activated by PHA in the presence of CsA restored the sensitivity to ATG and  $\text{F(ab')}_2$ -induced apoptosis (Fig 5B). Conversely, the addition of interferon- $\gamma$  (IFN- $\gamma$ ) restored T-cell proliferation,<sup>29</sup> but not the sensitivity to ATG-induced apoptosis. In agreement with previ-



**Fig 3. Expression of Fas-L mRNA induced by ATGs.** (Left) PBL were cultured in presence of medium alone, ATG no. 95-07 (100  $\mu\text{g/mL}$ ) or PHA (5  $\mu\text{g/mL}$ ) for 3 days. Dead cells were removed, and viable cells were stimulated with normal rabbit IgG at 100  $\mu\text{g/mL}$ , ATG no. 95-07 at 10  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  or PHA at 5  $\mu\text{g/mL}$  for 6 hours. (Right) Freshly isolated PBL were stimulated with normal rabbit IgG at 100  $\mu\text{g/mL}$  or ATG no. 95-07 at 10  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  for 6 hours. mRNA of each sample was amplified by RT-PCR as described in Materials and Methods with primers specific for actin or Fas-L. The number of amplification cycles selected within the exponential phase of PCR was 29 for actin and 32 for Fas-L. The PCR products were separated on 2% agarose gel and the PCR signal intensities were quantified by scanning the negative film. Results are expressed as the ratio of absorbance of Fas-L/absorbance of actin (mean  $\pm$  SEM of three experiments).

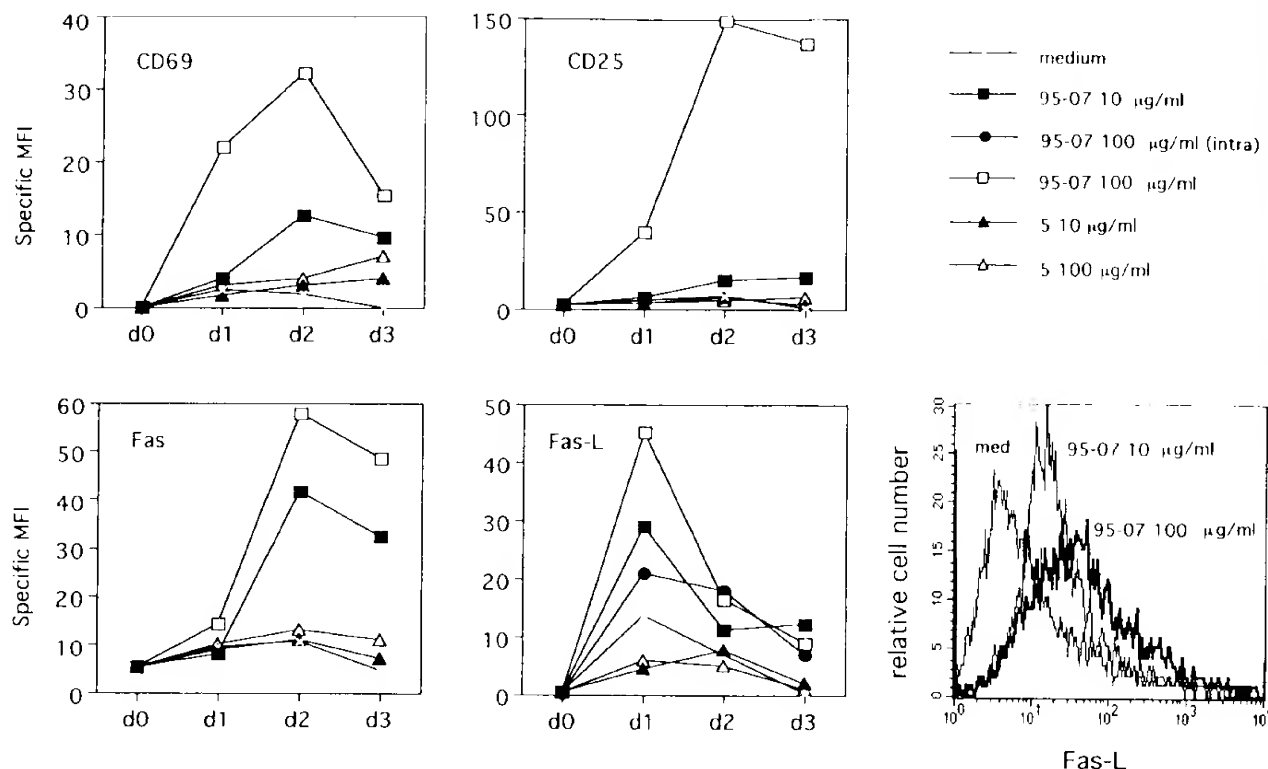


Fig 4. Effect of ATGs on CD69, CD25, Fas, and Fas-L surface expression. PBL were cultured in presence of medium alone or ATG no. 95-07 and ATG no. 5 at 10  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$  for 3 days. At days 0, 1, 2, and 3, surface expression of CD69, CD25, Fas, and Fas-L was determined by cytofluorometry. In parallel, incorporation of [ $^3\text{H}$ ]TdR uptake during the last 8 hours of culture was measured (med  $367 \pm 41$  cpm, ATG no. 5 10  $\mu\text{g/ml}$   $391 \pm 23$  cpm, ATG no. 5 100  $\mu\text{g/ml}$   $252 \pm 26$  cpm, ATG no. 95-07 10  $\mu\text{g/ml}$   $532 \pm 53$  cpm, and ATG no. 95-07 100  $\mu\text{g/ml}$   $11,500 \pm 103$  cpm). Histograms of Fas-L expression at day 1 are shown. Representative of four experiments with ATG no. 95-07 and of two with ATG no. 5.

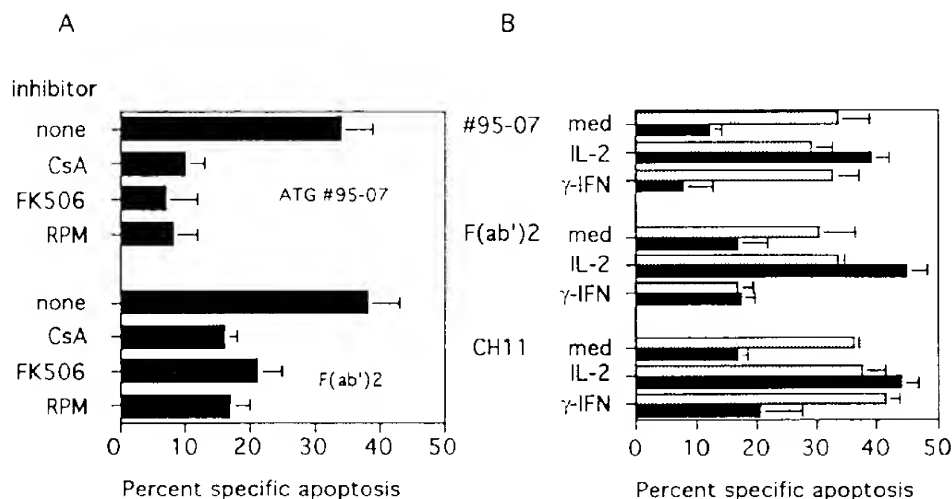


Fig 5. (A) Effect of immunosuppressive agents on ATG-mediated apoptosis. PBL were cultured for 3 days with PHA (5  $\mu\text{g/ml}$ ) and CsA (250 ng/mL), FK506 (10 nmol/L) or RPM (60 nmol/L) were added at the onset of the culture. Apoptosis was determined by fluorescence microscopy after staining with Hoechst 33342, 20 hours after treatment with ATG no. 95-07 or their F(ab')<sub>2</sub> fragments at 10  $\mu\text{g/ml}$ . (B) Effect of addition of exogenous IL-2 or IFN- $\gamma$ . PBL were cultured for 3 days with PHA (5  $\mu\text{g/ml}$ ); medium alone (gray bars) or CsA (250 ng/mL) (black bars) were added at the onset of the culture. Recombinant IL-2 (25 U/mL) or rIFN- $\gamma$  (500 U/mL) was added during the last 24 hours of activation. Apoptosis was determined by fluorescence microscopy after staining with Hoechst 33342, 20 hours after treatment with ATG no. 95-07, their F(ab')<sub>2</sub> fragments at 10  $\mu\text{g/ml}$  or the CH11 (1  $\mu\text{g/ml}$ ) MoAb. (Results are expressed as mean  $\pm$  SEM of three different experiments).

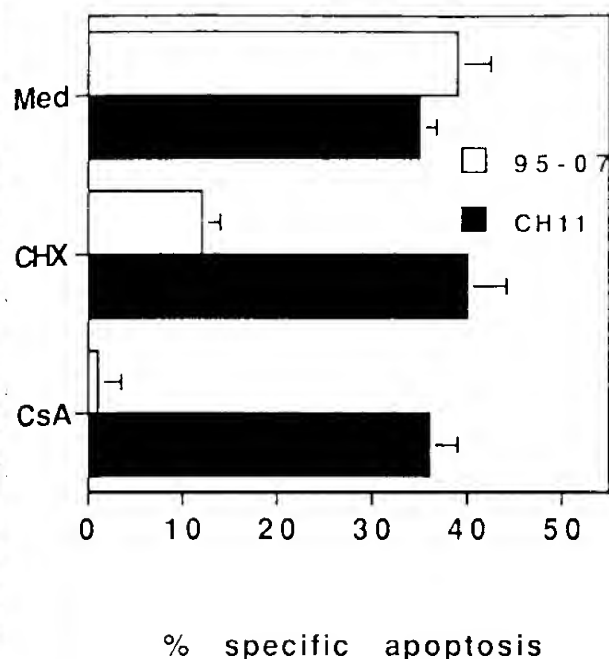


Fig 6. ATG-induced apoptosis is inhibited by CsA and requires protein synthesis. PBL were incubated for 3 days in the presence of PHA (5  $\mu$ g/mL). Dead cells were removed and viable cells were incubated for 3 hours with CsA (250 ng/mL) or CHX (0.5  $\mu$ g/mL) before treatment with ATG no. 95-07 (10  $\mu$ g/mL) or CH11 (1  $\mu$ g/mL). Apoptosis was determined by fluorescence microscopy after staining with Hoechst 33342. Results are expressed as mean  $\pm$  SD of three different experiments.

ous reports,<sup>29,30</sup> similar effects were observed as regards sensitivity to Fas-mediated apoptosis (Fig 5B).

Furthermore, CsA and FK506 were described as strongly inhibiting Fas-L expression in murine T-cell hybridomas.<sup>31</sup> Thus, we have tested whether incubation of 3-day PHA-activated PBL with CsA, just before ATG treatment would interfere with ATG-induced apoptosis. A 3-hour preincubation of PHA-blasts with CsA or CHX inhibited ATG-induced cell death but did not interfere with apoptosis induced by the anti-Fas MoAb (Fig 6). These data suggest that immunosuppressive agents that interfere with the IL-2 pathway can prevent ATG-induced apoptosis by inhibiting either Fas-L synthesis or the acquisition of sensitivity to Fas-L-mediated cell death by activated T cells.

*ATGs induce complement-mediated cytotoxicity at supramitogenic concentrations.* Binding of human C1q was measured by incubation of PBL in the presence of ATGs and fresh human serum, followed by flow cytometry assessment of the amount of bound C1q per cell. Heat-inactivated human serum was used as control. Maximal binding was achieved at 1 mg/mL. At lower ATG concentrations, only rabbit, but not equine, ATG bound C1q (Fig 7). C1q binding was comparable between resting PBL and preactivated cells.

The ability of ATGs to induce resting or PHA-activated PBMC lysis was evaluated in the presence of an exogenous source of human complement. Minimal cytotoxicity was observed at 10  $\mu$ g/mL with equine ATG, whereas maximal cytotoxicity was only achieved at very high concentrations (1 mg/mL) of ATGs. As a positive control of complement-mediated cytotoxicity, we used the CAMPATH-1M MoAb, which, in agreement with a

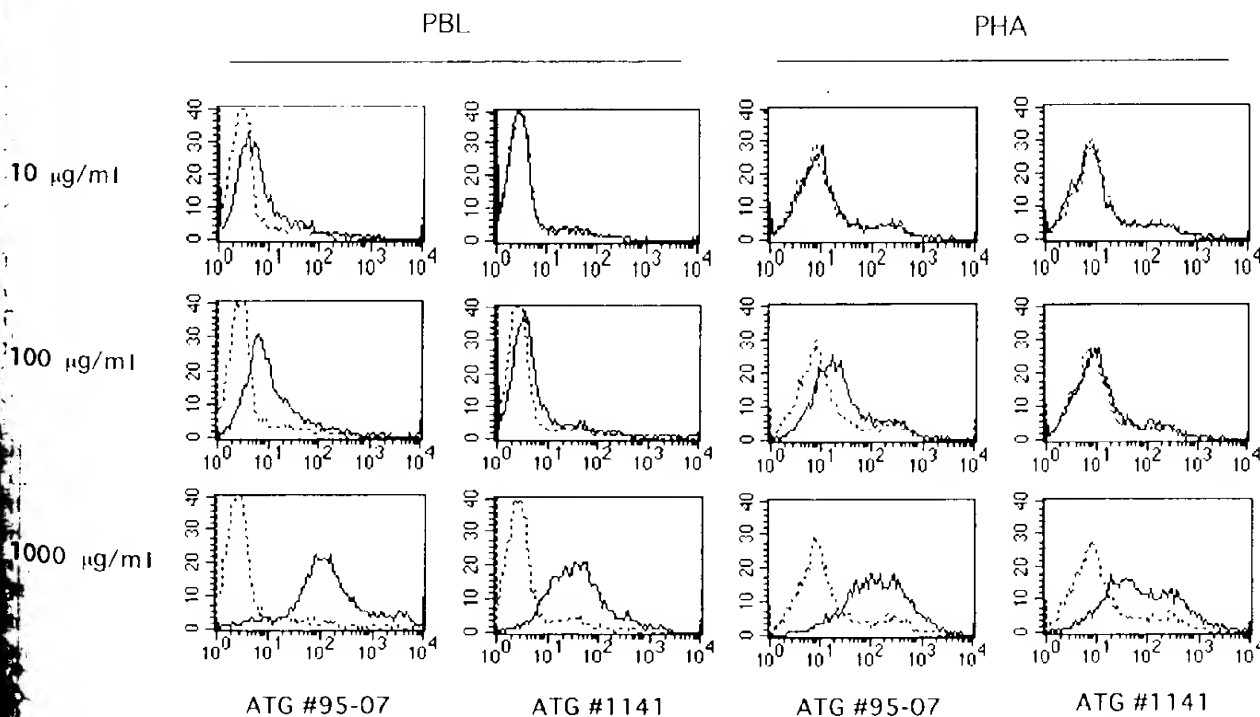


Fig 7. C1q binding to PBL or PHA-blasts sensitized with ATGs. PBL or PHA blasts were labeled with increasing amount of rabbit ATG (no. 95-07) or horse ATG (no. 1141) and then with autologous serum (solid line) or heat-inactivated serum as control (dashed line). C1q binding was detected by using FITC-goat anti-C1q polyclonal antibody and cell analyzed by flow cytometry as described in Materials and Methods. Representative of three independent experiments.

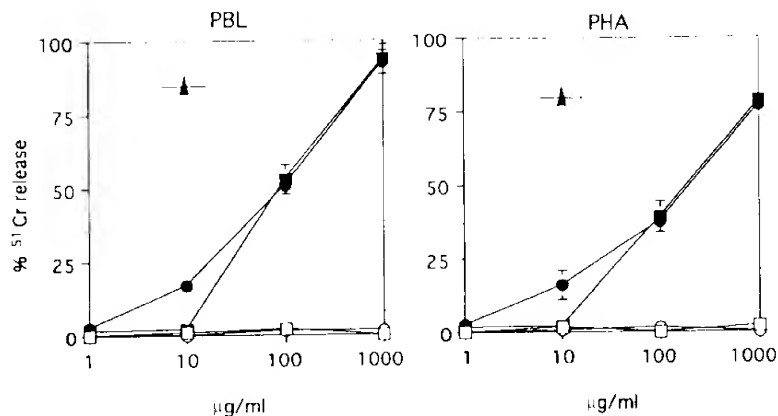


Fig 8. Complement-mediated lysis of PBMC versus PHA-Blasts. PBMC or 3-day PHA-activated PBMC were labeled with  $^{51}\text{Cr}$  and incubated with rabbit ATG (no. 95-07) (■), horse ATG (no. 1141) (●), control horse (○) or rabbit IgG (□) or the anti-CD52 MoAb CAMPATH-1M (IgM) (▲) at the indicated concentrations, for 30 minutes at 37°C, in the presence of 10% autologous serum. Results are expressed as specific release as defined in Materials and Methods (mean  $\pm$  SEM of three different experiments).

previous report,<sup>32</sup> induced about 80% lysis at 10  $\mu\text{g/mL}$ . Of note, no difference was observed, whether ATGs were obtained from horse (no. 1141) or rabbit (no. 95-07), and whether resting or PHA-activated PBMC were used as target cells in the complement-dependent lysis assay (Fig 8).

**ATGs induce antibody-dependent cell cytotoxicity at low concentrations.** ATGs no. 95-07 and no. 1141 were tested for their ability to induce ADCC of both resting and PHA-activated PBMC. We observed that this effect was concentration dependent, with a maximal cytotoxicity at 1  $\mu\text{g/mL}$  of ATG no. 95-07 and effective only when PHA-activated PBMC were used as target cells (Fig 9). As expected, the ADCC phenomenon was not observed with  $\text{F(ab')}_2$  fragments of ATG no. 95-07 and was restricted to ATG from rabbit origin, because ATG no. 1141 did not induce cell lysis at concentrations ranging from 0.01 to 100  $\mu\text{g/mL}$ .

## DISCUSSION

Both horse antilymphocyte globulins and rabbit ATGs are still used in the treatment of severe aplastic anemia, organ allograft rejection, and graft-versus-host disease (GVHD), but their mechanisms of action remain largely unknown. A major common feature of ATG treatment is peripheral lymphocyte depletion,<sup>1,4,5,33</sup> which usually persists throughout the administration period and slowly reverses thereafter. Although not formally demonstrated in clinical studies, lymphocyte depletion is likely to account for the immunosuppressive activity of ATGs.<sup>34</sup> The present study addressed the mechanisms of

peripheral lymphocytopenia, with special emphasis on the differential susceptibility of preactivated T cells (PHA blasts) versus nonactivated T cells to ATG-induced cell death. ATGs contain multiple antibody specificities with little batch-to-batch variability despite the use of different cell sources (thymocytes, T-cell lines, or B-cell lines) and different immunization protocols.<sup>6-8</sup> We therefore tested two ATG preparations of horse anti-human lymphocyte globulins (no. 1141) and rabbit antithymocyte globulins (no. 95-07) currently used in organ and bone marrow transplantation, as well as one horse ATG preparation (no. 5) previously used in kidney transplantation (selected because of its highly unusual lack of mitogenic activity related to the absence of demonstrable CD2 and CD3 specificities).<sup>7</sup> Horse anti-lymphocyte globulins are administered at 10 to 15 mg/kg/d,<sup>35</sup> and rabbit ATGs at 1.0 to 1.2 mg/kg/d, resulting in average serum levels of 0.5 mg/mL and 80 to 200  $\mu\text{g/mL}$ , respectively.<sup>5</sup> These dosages have been selected mostly on empiric grounds, but individual dosage adjustment to maintain absolute T-cell numbers of 50 to 100 cells/ $\mu\text{L}$  did not result in a major decrease in daily doses.<sup>35</sup> It is worth noting that the 10-fold dosage difference between equine and rabbit ATGs is not paralleled by differences in either specific antibody titers (eg, CD2, CD3, CD4, CD8)<sup>7</sup> or in vitro functional properties such as T-cell activation<sup>5,6,35,36</sup> or B-cell apoptosis.<sup>27</sup>

Complement-dependent lysis is initiated by the binding of human C1q to ATG-coated cells. At low and intermediate ATG concentrations, C1q binding was demonstrable with rabbit ATG on both PBL and PHA blasts but remained borderline or not

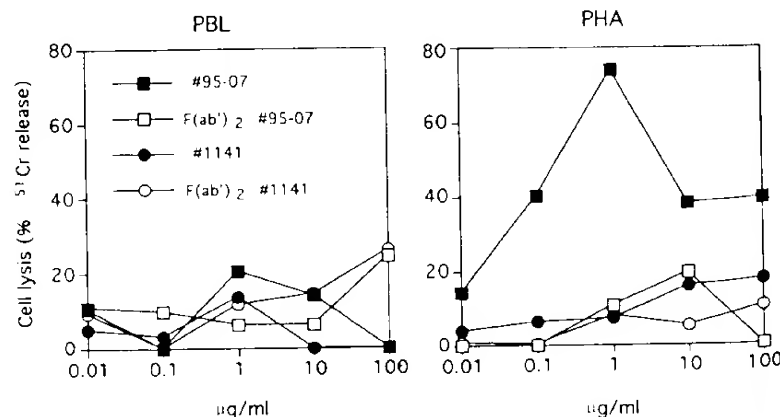


Fig 9. Antibody-dependent cell cytotoxicity of PBMC versus PHA-blasts. PBMC or 3-day PHA-activated PBMC were labeled with  $^{51}\text{Cr}$  and incubated with rabbit ATG (no. 95-07), horse ATG (no. 1141), or their  $\text{F(ab')}_2$  fragments at the indicated concentrations in presence of effector cells for 6 hours at 37°C. Results are expressed as specific release as in Fig 8. Representative of two independent experiments.

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detectable with equine ATG (Fig 7). As shown with chimeric monoclonal antibodies of different isotypes, C1q binding may not be correlated with cell lysis.<sup>37</sup> Therefore, we used the highly sensitive chromium release assay to measure complement-dependent lysis. The data (Fig 8) indicate that equine and rabbit ATGs are equally effective on PBMC and PHA blasts, but only at high concentrations. In keeping with our observation, complement consumption, as measured by decreased serum CH50 activity, was recorded in some patients during equine ATG treatment, but never with rabbit ATG (Y. Lebranchu, personal communication, January 1997).

ADCC has been suggested as a possible mechanism of lymphocyte depletion by ATG.<sup>1,5</sup> NK cells present in peripheral blood are potent effectors of Fc receptor-dependent cell lysis. Our results indicate that only PHA blasts, but not PBMC, can be lysed through an ADCC mechanism, suggesting that rabbit ATGs could display some selectivity toward preactivated lymphocytes, should a similar mechanism operate *in vivo*. Equine ATG on the other hand was completely ineffective in this assay.

The major homeostatic mechanism that prevents lymphoid tissue hyperplasia despite repeated antigenic stimulations and T- or B-cell clonal expansion is activation-induced cell death (AICD) mediated by Fas/L-Fas interaction.<sup>38</sup> We therefore investigated the possible contribution of the Fas pathway in ATG-induced lympholysis. Fas-L is constitutively expressed in a variety of tissues, including immunologically privileged sites (eg, eye, Sertoli cells), some tumors,<sup>39,40</sup> and monocytes<sup>41</sup> and produced by a subset of T cells after repeated activation through the TCR/CD3 or CD2 pathways, or both.<sup>42</sup> Knowing the T-cell mitogenic properties of ATGs,<sup>35,36</sup> we were not surprised to observe that restimulation by ATGs of PBL preactivated by various mitogens, including ATGs themselves, triggered Fas-L gene expression (Fig 3). However, quite unexpectedly, ATGs were also found to induce Fas-L mRNA and protein expression in nonpreactivated PBL, even at low concentrations (10 µg/mL) sufficient to trigger CD69, but not CD25, expression and therefore remain below the mitogenic threshold (Fig 4). Although they express Fas receptors, these CD25 negative cells do not respond to IL-2 and therefore cannot become sensitive to Fas-dependent apoptosis, as discussed below. The fact that blocking Fas/Fas-L interaction completely suppressed ATG-induced apoptosis (Fig 2) provides unequivocal evidence for a role of the Fas pathway in ATG-mediated lymphocyte cell death. Target cells for Fas-L should not only express Fas receptors that are rapidly induced upon activation, but should also become sensitive to Fas-mediated apoptosis, a property that is strictly dependent on an IL-2 signal.<sup>29,30</sup> Hence pharmacological interference with the IL-2 pathway in activated T cells, by the addition of CsA, FK506, or rapamycin, prevents Fas-positive cells from becoming sensitive to ATG- and to Fas-L- (or agonist anti-Fas antibody)-dependent apoptosis. CsA also inhibits Fas-L expression.<sup>31,43</sup> Therefore, concomitant administration of ATGs with any immunosuppressive agent that interferes with the IL-2 pathway (eg, CsA, FK506, rapamycin, CTLA-4 Ig, or CD25 antibodies) is likely to prevent Fas-dependent ATG-induced lymphocyte depletion. Furthermore, this mechanism of lymphocyte apoptosis may be impaired in clinical situations associated with high plasma levels of soluble Fas.

In conclusion, this *in vitro* study describes some of the

mechanisms that may account for lymphocyte depletion during ATG therapy. However, one should keep in mind that opsonization and subsequent phagocytosis by spleen, liver, and lung macrophages is likely to account for the massive and rapid lymphocytopenia observed with the current protocols. Nevertheless, other mechanisms should be considered, some of which could represent a therapeutic objective in the design of future protocols aimed at a more selective immunosuppression. Complement-dependent lysis does not discriminate between resting and preactivated T cells. Because it is achieved at high ATG concentrations, it may occur in treatment with horse ATG, but this is less likely with rabbit ATG. In this respect, the relevance of complement-dependent lymphocytotoxicity for the standardization of ATG preparations is questionable. Serum ATG concentrations achieved with current dosages are mitogenic for peripheral T cells. Hence, they could trigger Fas-L expression and induce sensitivity to Fas-L in the vast majority of T cells, unless CsA or FK506 that block these processes is administered concomitantly. An important finding of this study is that some ATG at low, submitogenic concentrations may trigger Fas-L expression, resulting in the selective death of preactivated, but not resting, lymphocytes. An ATG preparation (no. 5) lacking mitogenic activity, and with no demonstrable CD2 and CD3 specificities, was devoid of this property, suggesting that "lymphocyte activating" antibodies (eg, CD2, CD3) may be critical in achieving Fas-dependent apoptosis. Similarly, ADCC that also occurs at low rabbit ATG concentration selectively targets activated, but not resting, T cells. These properties could be used in protocols aiming at the selective elimination of *in vivo* activated T cells (eg, donor-specific alloreactive T cells in organ transplantation, recipient-specific T cells in GVHD), while sparing nonactivated T cells. Such protocols would require much lower doses than those currently used, in order to maintain serum ATG concentrations within a 10- to 20-µg/mL range, instead of 100 µg/mL. Their feasibility will be evaluated in the cynomolgus monkey and, depending on the outcome of these experiments, clinical trials may be considered.

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# Non-Host-Reactive Donor CD8<sup>+</sup> T Cells of Tc2 Phenotype Potently Inhibit Marrow Graft Rejection

By Daniel H. Fowler, Bernard Whitfield, Michael Livingston, Paul Chrobak, and Ronald E. Gress

Donor CD8<sup>+</sup> T cells capable of host reactivity inhibit marrow graft rejection, but also generate graft-versus-host disease (GVHD). To evaluate whether the Tc1- and Tc2-type subsets of CD8 cells might inhibit rejection without host reactivity, we established an F1 into-parent murine bone marrow transplant model. Donor Tc1 and Tc2 cells were generated that preferentially secreted type I or type II cytokines; both subsets possessed potent cytolytic function, and clonally deleted host-type allospecific precursor CTL in vitro. B6 hosts receiving 950 cGy irradiation did not reject the donor marrow (F1 chimerism of 78.6%; n = 10), whereas hosts receiving 650 cGy rejected the donor marrow (3.8% chime-

ism; n = 8). At 650 cGy irradiation, the addition of Tc2 cells to the F1 marrow resulted in extensive F1 chimerism (70.8%) in 8 of 8 recipients; in contrast, alloengraftment was not consistently observed in mice receiving Tc1 cells or unmanipulated CD8 cells. Furthermore, when the preparative regimen was further reduced to 600 cGy, only hosts receiving the Tc2-type cells did not reject the F1 marrow. We conclude that Tc2 cells potently inhibit marrow graft rejection without inducing an alloaggressive response and that non-host-reactive Tc2 cells therefore facilitate engraftment across genetic barriers with reduced GVHD.

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**T**HE HOST-VERSUS-GRAFT immune response, which results in graft rejection, is a primary limitation to the transfer of marrow across genetic barriers. The observation that recipients of T-cell-depleted transplants have a high rate of marrow failure<sup>1</sup> has led to the realization that donor T cells abrogate graft rejection; indeed, the presence of donor T cells in the marrow appears to be one of the primary determinants of whether alloengraftment occurs.<sup>2</sup> As such, administration of T-cell-enriched marrow is one approach to the facilitation of alloengraftment. However, in addition to preventing graft rejection, donor T cells generate an alloaggressive response against host antigens, which can result in graft-versus-host disease (GVHD). The donor CD8<sup>+</sup> T-cell subset appears to be particularly capable of abrogating rejection<sup>3</sup>; however, CD8<sup>+</sup> T cells also contribute significantly to the generation of GVHD.<sup>4,5</sup> (Given this limitation, we have evaluated whether functional subsets of donor CD8 cells might prevent graft rejection with reduced GVHD.)

Recently, the existence of cytokine-secreting subsets of cytotoxic CD8<sup>+</sup> T cells has been demonstrated<sup>6,7</sup>; the Tc1 subset secretes the type I cytokines interleukin-2 (IL-2) and interferon- $\gamma$  (IFN- $\gamma$ ), whereas the Tc2 subset secretes the type II cytokines IL-4, IL-5, and IL-10. Both cytokine-secreting subsets of CD8 cells possess cytolytic function, which has led to the Tc1/Tc2 terminology.<sup>7</sup> Such CD8 functional subsets appear to differentially mediate allogeneic responses; eg, we and others have observed that the Tc2 subset results in reduced GVHD.<sup>8,9</sup> Importantly, the Tc2 subset can also mediate a graft-versus-leukemia (GVL) effect.<sup>8,10</sup> These results suggest that Tc2 cells might represent a CD8 population capable of mediating beneficial allogeneic responses (such as the mediation of GVL effects or the abrogation of graft rejection) with reduced detrimental effects (less severe GVHD). In light of these observations, we have compared the Tc1 and Tc2 subsets of CD8<sup>+</sup> T cells for their ability to prevent marrow rejection.

To study the engraftment effects of the Tc1 and Tc2 subsets of donor CD8 cells independent of their GVHD effects, we have established an F1 into-parent model of graft rejection (B6C3F1 bone marrow into sublethally irradiated B6 hosts). In this type of rejection model, donor CD8 cells share the haplotype of the parental host and thus do not induce an alloaggressive reaction against the host; T-cell-mediated facilitation of engraftment in such models has been attributed to a veto effect.<sup>11</sup> In the veto

effect, host-type precursor CTL capable of mediating rejection are clonally deleted by cytotoxic donor cells that express the alloantigens present on the marrow graft<sup>12</sup>; in addition, the donor cells mediating the clonal deletion are nonreactive to host antigens. The definition of veto cells is therefore a functional one, and multiple cell types have been observed to possess veto-type activity in the setting of murine allogeneic bone marrow transplantation, including bone marrow-derived natural killer cells<sup>13</sup> and CD4<sup>+</sup><sup>14</sup> and CD8<sup>+</sup><sup>3,14</sup> T cells. Previous experiments have demonstrated that the perforin/granzyme pathway of cytotoxicity is important in the mediation of the veto effect.<sup>12</sup> Thus, in light of studies that indicate that the Tc2 subset of CD8<sup>+</sup> T cells preferentially uses the perforin/granzyme pathway,<sup>15</sup> we hypothesized that non-host-reactive Tc2 cells would prevent marrow rejection.

In this study, we compared the Tc1 and Tc2 subsets of donor CD8<sup>+</sup> T cells for their ability to facilitate alloengraftment and have determined that the Tc2 subset potently inhibits marrow graft rejection. By using an F1 into-parent model, we have shown that Tc2-mediated abrogation of rejection can occur independent of an alloaggressive response. The administration of non-host-reactive Tc2 cells therefore represents a strategy for abrogating rejection with reduced GVHD and thus may allow for the transfer of marrow across genetic barriers.

## MATERIALS AND METHODS

*In vitro* generation of donor CD8<sup>+</sup> T cells of Tc1 and Tc2 phenotype. Spleen cells from donor B6D2F1 mice (C57BL/6  $\times$  DBA/2, H-2<sup>b/d</sup>), obtained from Frederick Cancer Research Facility (Frederick, MD) and

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used at 8 to 16 weeks of age) were harvested, lysed in Tris-ammonium chloride buffer (Biofluids, Rockville, MD), and brought to a concentration of  $4 \times 10^7$  cells/mL in RPMI 1640 (Life Technologies, Grand Island, NY) containing 10% fetal calf serum (FCS; HyClone, Logan, UT). This splenic single-cell suspension was enriched for T cells (goat antimouse H and L bioparticles; PerSeptive Diagnostics, Cambridge, MA) and then enriched for CD8 cells by complement treatment (rabbit low-tox; Cedarlane, Hornby, Ontario, Canada) after incubation with anti-CD4 (supernatant from clone RL172/4<sup>16</sup>). The CD8-enriched spleen cells were then resuspended at  $1 \times 10^6$ /mL and cocultured at a ratio of 1:4 with irradiated (3,000 cGy) whole spleen cells from B6C3F1 mice (C57Bl/6  $\times$  C3H/HeN, H-2<sup>b/k</sup>).

The coculture was performed in 75-cm<sup>2</sup> flasks (Costar, Cambridge, MA) in 40 mL of RPMI 1640 supplemented with 10% FCS, sodium pyruvate (1%), nonessential amino acids (1%), L-glutamine (0.5%), 2-ME ( $5 \times 10^{-5}$  mol/L), penicillin (0.5%), streptomycin (0.5%), and N-acetyl cysteine (Sigma Chemical Co, St Louis, MO; 10 mmol/L, pH adjusted to 7.2). All cocultures received recombinant human IL-2 at 40 Cetus units (CU)/mL (kindly provided by Dr Martin Giedlin, Chiron Therapeutics, Emeryville, CA) and recombinant human IL-7 (20 ng/mL; Peprotech, Rocky Hill, NJ) on days 0 and 2. Tc1 cultures were supplemented (day 0) with recombinant murine IL-12 (20 U/mL; kindly provided by Dr Stan Wolf, Genetics Institute, Cambridge, MA) and recombinant human transforming growth factor  $\beta$ -one (TGF- $\beta$ ; 10 ng/mL; R&D Systems, Minneapolis, MN), whereas Tc2 cultures were supplemented (day 0) with recombinant murine IL-4 (1,000 U/mL; Peprotech). On day 5 of culture, Tc1 and Tc2 flasks were harvested, brought to a final concentration of  $0.5 \times 10^6$ /mL in 40 mL of fresh media, and restimulated with irradiated (3,000 cGy) B6C3F1 spleen cells at a ratio of 1:4. At the time of restimulation, IL-2 (40 U/mL) and IL-7 (20 ng/mL) were added to both Tc1 and Tc2 cultures.

**Flow cytometric (FCM) evaluation of Tc1/Tc2 surface phenotype.** To evaluate the Tc1 and Tc2 populations for cell surface phenotype, aliquots from the CD8 cultures were harvested on day 7, washed, and resuspended in FCM media consisting of Hanks' balanced salt solution (HBSS; Life Technologies) supplemented with 0.5% bovine serum albumin (BSA; Sigma) and 0.1% azide. Cells were first incubated with unlabeled anti-Fc receptor (2.4G2; PharMingen, San Diego, CA) and then stained with anti-CD4 fluorescein isothiocyanate (FITC) and anti-CD8 phycoerythrin (PE) (Becton Dickinson Immunocytometry Systems [BDIS], Mountain View, CA); negative control stains consisted of Leu8 FITC and Leu4 PE. Cells were also stained with anti-CD69 PE (PharMingen). Two-color flow cytometry was performed on a FACSort (BDIS) using LYSIS II software. Five thousand to 10,000 live events were acquired for analysis; dead cells were gated out on the basis of propidium iodide staining.

**Cytokine secretion profiles of Tc1 and Tc2 populations by enzyme-linked immunosorbent assay (ELISA).** On day 7 of culture, aliquots from Tc1 and Tc2 cultures were harvested, brought to a final concentration of  $0.5 \times 10^6$ /mL, and stimulated in 24-well plates (Costar) with either syngeneic B6D2F1 or semiallogeneic B6C3F1 spleen cells (irradiated 3,000 cGy; 1:4 ratio). Supernatants were harvested after 24 hours and tested in two-site ELISAs using commercially available reagents (purified and biotinylated anti-cytokine antibody pairs; PharMingen). Cytokine levels were calculated by reference to standard curves constructed on supernatants containing known amounts of recombinant cytokine.

**Evaluation of Tc1/Tc2 cytolytic function.** On day 7 of culture, aliquots from Tc1 and Tc2 cultures were harvested and tested for their ability to lyse the allogeneic tumor line P210<sup>17</sup> (H-2<sup>b</sup>; myeloid line transfected with the *ber/abl* oncogene, kindly provided by Dr James Griffin, Dana-Farber Cancer Institute, Boston, MA). The syngeneic control target EL-4 (H-2<sup>b</sup>; American Type Tissue Culture TIB 39) was used to determine allospecificity of cytolytic function; as a positive control for this syngeneic target, CD8<sup>+</sup> T cells from DBA mice were

stimulated under Tc1 and Tc2 conditions using spleen cells from mice (H-2<sup>b</sup>) as stimulator cells. Standard chromium-release assays performed, with calculation of the percentage of specific lysis.<sup>18</sup>

**In vitro assay of Tc1- and Tc2-mediated deletion of precursor.** Using a previously described in vitro model of veto cell function, tested the Tc1 and Tc2 populations for their ability to clonally delete allospecific precursor CTL. In this model, a mixed lymphocyte reaction was established using a 10:1 mixture of responder spleen cells (C57Bl/6(H-2<sup>b</sup>) and C57Bl/6 transgenic mice (2C mice; CD8<sup>+</sup> T<sub>H</sub> transgenic at the TCR locus for I-E allospecificity<sup>19</sup>). Responder cells ( $4 \times 10^6$ ) were stimulated in 24-well plates (Costar) with irradiated spleen cells (2,500 cGy) from DBA/2 mice (H-2<sup>d</sup>). Expansion of allospecific CD8<sup>+</sup> T cells was monitored by daily cell count determinations and flow cytometry (the transgenic TCR was identified by flow cytometry after staining with directly FITC-labeled 1B2 antibody specific for the transgenic TCR<sup>19</sup>). In this culture system, transgenic CD8<sup>+</sup> T cells undergo an approximate 10-fold expansion between days 2 and 3 of the MLR. To evaluate the ability of CD8 functional T<sub>H</sub> subsets to mediate veto activity in vitro, Tc1 and Tc2 populations were generated (as described above) and added to the transgenic cultures on day 2; on day 3, cell counting and flow cytometry was performed to determine transgenic CTL yield. The Tc1 and Tc2 populations were generated from CD8<sup>+</sup> T cells from B6D2F1 mice and thus shared the H-2<sup>b</sup> haplotype with the transgenic CTL and shared the H-2<sup>d</sup> haplotype with the irradiated stimulator cells. As such, Tc1- or Tc2-mediated deletion of the transgenic CTL may occur by a veto-type mechanism.

**F1 into-parent transplantation model.** Parental B6 mice receive total body irradiation (<sup>137</sup>Cs  $\gamma$  radiation source, 101 cGy/min; Gammacell 40; Atomic Energy of Canada, Ltd, Ottawa, Ontario, Canada) depending on the particular experiment, the radiation dose varied from 600 to 950 cGy. Four to 6 hours after irradiation, all mice received an intravenous injection of  $1 \times 10^7$  T-cell-depleted (TCD) bone marrow cells from B6D2F1 mice (antibody/complement depletion using the anti-T-cell monoclonal HO-13-2<sup>20</sup>). Control mice received only the TCD marrow, whereas recipients in other groups also received a separate intravenous injection of  $1 \times 10^7$  CD8<sup>+</sup> T cells of donor B6D2F1 origin; such CD8<sup>+</sup> T cells consisted of either in vitro generated CD8<sup>+</sup> T cells of Tc1 or Tc2 phenotype (harvested from flasks on day 7 of culture) or uncultured B6D2F1 CD8<sup>+</sup> T cells (prepared by T-cell enrichment and anti-CD4/complement treatment, as described above).

**Evaluation of donor chimerism posttransplant by flow cytometry.** Donor cell chimerism of transplant recipients for each experiment was determined by flow cytometry at approximately 1 month posttransplant and again after 3 months posttransplant. Heparinized peripheral blood was obtained from the retro-orbital sinus, and lymphocytes were isolated by density centrifugation (Cellsep; 1.077 specific gravity; Larex, Inc, St Paul, MN) and subsequently washed in FCM media. Cells were incubated with unlabeled anti-Fc receptor antibody (2.4G2; PharMingen) and stained with anti-H-2k<sup>b</sup> FITC and anti-H-2k<sup>d</sup> PE (PharMingen); cells were also stained with isotype control antibodies to define background staining. Lymphocytes taken from untreated B6 and B6D2F1 mice were used to define positive and negative quadrants. Other stains used to evaluate lineages of engraftment were anti-CD19 FITC, anti-granulocyte FITC, and anti-Thy 1.2 FITC (all from PharMingen).

**Statistical methodology.** *P* values were obtained using the two-sided matched-rank analysis of Wilcoxin; values less than .05 were considered statistically significant.

## RESULTS

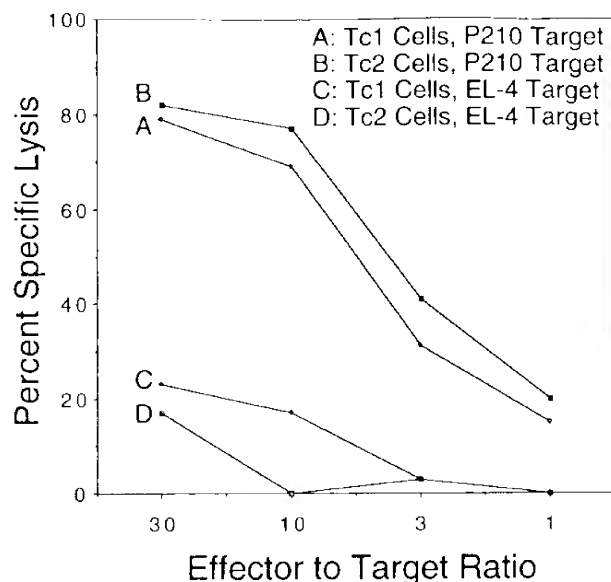
**Phenotyping of in vitro-generated donor CD8<sup>+</sup> T cells of Tc1 and Tc2 phenotype.** After 7 days, cells from the Tc1 and Tc2 cultures were phenotyped by flow cytometry. Both Tc1 and Tc2 culture conditions resulted in a population that was greater than

90% CD8<sup>+</sup>, with less than 2% contaminating CD4<sup>+</sup> cells. Similar to results we have reported previously,<sup>8</sup> cells from the Tc2 culture had lower surface CD8 expression relative to the Tc1 culture (mean fluorescence intensity of CD8 expression for Tc2 cells was 3,662, whereas Tc1 cell mean fluorescence intensity of CD8 expression was 5,131). Also, expression of surface CD69 was measured to compare the Tc1 and Tc2 cultures for their activation status<sup>21</sup>; the majority of cells (>85%) in both Tc1 and Tc2 cultures were positive for CD69, indicating that both populations were similarly activated at the time of their *in vivo* evaluation (day 7 of culture).

On day 7 of culture, cells from the Tc1 and Tc2 cultures were harvested and evaluated for cytokine phenotype. To evaluate the allospecific cytokine secretion pattern, cultured CD8 cells were restimulated with either syngeneic B6D2F1 spleen cells or semiallogeneic B6C3F1 cells; cytokine secretion was allospecific, because stimulation of Tc1 and Tc2 cells with syngeneic spleen cells did not result in significant cytokine production above the detection limits of the assays (IL-2 assay, 0.8 CU/mL; IFN- $\gamma$ , 1.0 IU/mL; IL-4, 20 pg/mL; IL-5, 320 pg/mL; and IL-10, 40 pg/mL). In response to restimulation with the third-party alloantigen (H-2<sup>b</sup>), cells from the Tc1 culture secreted the type I cytokines IL-2 (10.9 CU/mL) and IFN- $\gamma$  (152 IU/mL), but did not secrete the type II cytokines. In marked contrast, cells from the Tc2 culture secreted the type II cytokines IL-4 (204 pg/mL), IL-5 (3,193 pg/mL), and IL-10 (1,510 pg/mL) and secreted reduced levels of the type I cytokines IL-2 (1.2 CU/mL) and IFN- $\gamma$  (19.9 IU/mL).

Cytolytic function of the Tc1 and Tc2 cultures was evaluated in chromium release assays using the allogeneic tumor target, P210; this target shares the alloantigen (H-2<sup>b</sup>) used for the *in vitro* CD8 generation. As Fig 1 shows, CD8<sup>+</sup> T cells secreting either type I or type II cytokines were similarly effective in their lysis of the allogeneic P210 target. In contrast, the Tc1 and Tc2 populations mediated only nominal lysis of the syngeneic EL-4 target; the ability of the EL-4 control target to be lysed was confirmed by generating Tc1- and Tc2-type effector cells of anti-H-2<sup>b</sup> specificity (both populations showed greater than 65% specific lysis of the EL-4 target at a 30:1 E:T ratio). Thus, the Tc1 and Tc2 populations were allospecific both in their cytokine secretion and cytolytic function. As such, the *in vitro* culture methodology was effective in generating allospecific CD8<sup>+</sup> donor T cells of Tc1 and Tc2 phenotype.

**Both Tc1- and Tc2-type populations effectively delete precursor CTL *in vitro*.** Graft rejection is mediated in part by radioresistant allospecific CTL<sup>22-25</sup>; the clonal deletion of such CTL may be one mechanism whereby donor T cells abrogate graft rejection. A model has been developed to evaluate the ability of cytotoxic cells to clonally delete allospecific CTL *in vitro* by a veto-type mechanism<sup>12</sup>; using this model, we compared the Tc1 and Tc2 populations for their ability to clonally delete precursor CTL. In this model, the fate of allospecific precursor CTL is determined by measuring the flow cytometric expression of the transgenic TCR expressed by these CTL. It is important to note that, similar to the *in vivo* graft rejection model, this *in vitro* model is a one-way alloreactive system; ie, the transgenic precursor CTL can recognize the stimulator cells and the Tc1 or Tc2 populations, but the Tc1 and Tc2 cells are syngeneic relative to the precursor CTL. Figure 2



**Fig 1.** Cytolytic function of the Tc1 and Tc2 populations. CD8<sup>+</sup> T cells from B6D2F1 donor mice were stimulated *in vitro* with irradiated spleen cells from B6C3F1 mice under Tc1 or Tc2 conditions, harvested on day 7 of culture, and plated in a standard 4-hour chromium-release assay at the stated E:T ratios with the allogeneic tumor target, P210 (H-2<sup>b</sup>), or the syngeneic tumor target, EL-4 (H-2<sup>k</sup>). Each data point was performed in triplicate, with less than 5% standard deviation for each point.

shows that both Tc1 and Tc2 cells were similarly effective in their ability to delete the allospecific precursor CTL population; the addition of  $1 \times 10^6$  Tc1 or Tc2 cells on day 2 of the MLR resulted in an approximate one log reduction in transgenic CTL number by day 3 of culture.

**F1 CD8<sup>+</sup> T cells of Tc2 phenotype potently inhibit marrow graft rejection.** Having generated CD8<sup>+</sup> T cells of both Tc1 and Tc2 phenotype from F1 donors, we next evaluated their effect on F1 marrow engraftment in an F1 into-parent model of graft rejection. B6 mice were sublethally irradiated and subsequently received marrow from B6D2F1 mice; control mice received only the F1 marrow, whereas other treatment groups received additional donor CD8<sup>+</sup> T cells of Tc1 or Tc2 phenotype. Figure 3 shows the flow cytometry result for determination of F1 chimerism at both day 30 and day 90 posttransplant. Irradiation of the B6 hosts with a high dose of irradiation (950 cGy) resulted in F1 marrow engraftment (the range of F1 chimerism was 56% to 76% at day 30 and 92% to 95% at day 90). In contrast, B6 hosts that were irradiated at a lower dose (650 cGy) displayed nearly complete host-type chimerism by day 90 posttransplant (<2% F1 chimerism), thus indicating rejection of the F1 marrow. In this experiment, hosts prepared with 650 cGy irradiation that received marrow enriched with F1 CD8<sup>+</sup> T cells of Tc1 phenotype also rejected the F1 marrow (<3% chimerism in all recipients). In marked contrast, hosts prepared with 650 cGy irradiation that received additional CD8<sup>+</sup> T cells of Tc2 phenotype had predominately donor-type chimerism (the range of F1 chimerism was 51% to 66% at day 30 and 82% to 91% at day 90). Other flow cytometry data (not shown) indicated that the chimerism occurred in the T-cell, B-cell, and granulocyte lineages. This experiment therefore

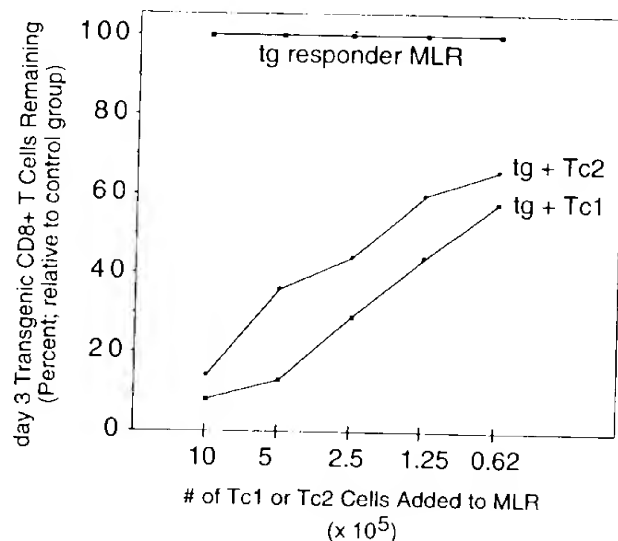


Fig 2. Both Tc1 and Tc2 populations clonally delete precursor CTL in vitro by a veto-type mechanism. A mixed lymphocyte reaction was established in 24-well plates using a 10:1 mixture of responder spleen cells from C57Bl/6(H-2<sup>b</sup>) and C57Bl/6 transgenic mice (2C mice; CD8<sup>+</sup> T cells are transgenic at the TCR locus for L<sup>d</sup> allospecificity) and stimulator spleen cells from DBA/2 mice (H-2<sup>d</sup>). The yield of transgenic CD8<sup>+</sup> T cells in the MLR (tg responder MLR) was calculated by determination of cell counts and transgene percentage (transgenic TCR was identified by flow cytometry after staining with FITC-labeled 1B2 antibody); in this system, the transgenic CD8 population expands approximately 10-fold between days 2 and 3 of the MLR. To evaluate the ability of Tc1- and Tc2-type cells to clonally delete this transgenic population by a veto mechanism, CD8<sup>+</sup> T cells from B6D2F1 donor mice were stimulated in vitro with irradiated spleen cells from B6C3F1 mice under Tc1 or Tc2 conditions, harvested on day 7 of culture, and added to the transgenic MLR at the indicated numbers per well (tg + Tc1 and tg + Tc2) on day 2 of the MLR. The yield of transgenic CD8 cells was then determined on day 3 of the MLR.

indicated that the Tc2-type cells had a marked ability to prevent acute marrow graft rejection and that Tc2-mediated facilitation of engraftment resulted in long-term, stable marrow engraftment.

To further evaluate the effect of the Tc1 and Tc2 populations

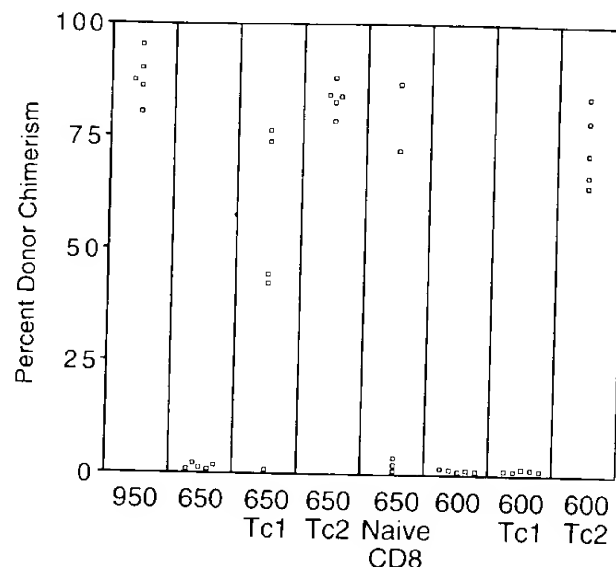


Fig 4. Tc2-type donor CD8<sup>+</sup> T cells are enriched in their ability to abrogate marrow graft rejection. Host B6 (H-2<sup>b</sup>) mice were irradiated at 950, 650, or 600 cGy; all mice received  $1 \times 10^7$  TCD bone marrow cells from B6D2F1 (H-2<sup>b/d</sup>) donor mice. Engraftment control mice (950/-) and rejection control mice (650/-) received only the donor bone marrow at the time of transplantation; other groups received additional in vitro-generated donor CD8<sup>+</sup> T cells ( $1 \times 10^7$  cells) of Tc1-type (650/Tc1, 600/Tc1) or Tc2-type (650/Tc2, 600/Tc2) or additional unmanipulated donor CD8 cells (650/naive CD8). Each treatment group consisted of 5 mice. Peripheral blood lymphocytes were isolated on day 41 posttransplant and stained with H-2<sup>b</sup> FITC (common to both donor and host cells) and H-2<sup>d</sup> PE (specific for donor cells); the percentage of donor and host chimerism was then determined by flow cytometry.

on F1 marrow engraftment, B6 host mice were irradiated (950, 650, or 600 cGy) and received injections of either F1 marrow alone or marrow and F1 CD8 cells that were cultured in vitro under Tc1 or Tc2 conditions; a separate treatment group received F1 marrow and unmanipulated F1 CD8<sup>+</sup> T cells (naive CD8). As Fig 4 shows (chimerism results on day 41 posttransplant), hosts receiving 950 cGy irradiation displayed nearly

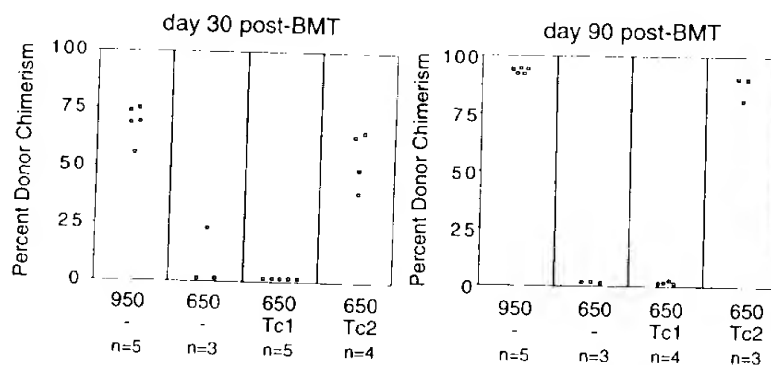
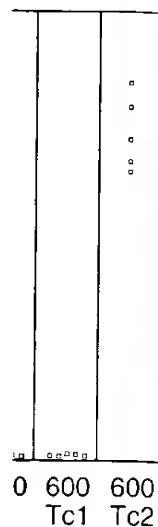


Fig 3. Tc2-type donor CD8<sup>+</sup> T cells abrogate marrow graft rejection. Host B6 (H-2<sup>b</sup>) mice were irradiated at 950 or 650 cGy; all mice received  $1 \times 10^7$  TCD bone marrow cells from B6D2F1 (H-2<sup>b/d</sup>) donor mice. Engraftment control mice (950/-) and rejection control mice (650/-) received only the donor bone marrow at the time of transplant; other groups received additional donor CD8<sup>+</sup> T cells ( $1 \times 10^7$  cells) of Tc1-type (650/Tc1) or Tc2-type (650/Tc2). Peripheral blood lymphocytes were isolated on days 30 and 90 posttransplant and stained with H-2<sup>b</sup> FITC (common to both donor and host cells) and H-2<sup>d</sup> PE (specific for donor cells); the percentage of donor and host chimerism was then determined by flow cytometry. Each data point represents the donor chimerism result for an individual animal.



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complete F1 engraftment, whereas hosts receiving 650 cGy irradiation uniformly rejected the F1 marrow. At 650 cGy of host irradiation, mice receiving the F1 marrow and CD8<sup>+</sup> T cells of Tc1 phenotype displayed a variable level of F1 engraftment (F1 chimerism of 0.6%, 42%, 44%, 73%, and 76%); thus, in contrast to the experiment shown in Fig 3, donor CD8 cells of Tc1-type were capable of abrogating the marrow graft rejection response. The administration of unmanipulated F1 CD8 cells also did not result in consistent F1 engraftment (F1 chimerism of 0.7%, 3%, 8%, 71%, and 86%). Thus, at 650 cGy host irradiation, donor T cells of naive or Tc1 phenotype partially abrogated the graft rejection response. In contrast, mice receiving F1 marrow and Tc2-type CD8 cells had a high level of F1 chimerism in 5 of 5 recipients at the 650 cGy dose of irradiation (F1 chimerism of 78%, 82%, 83%, 84%, and 88%). When the results shown in Figs 3 and 4 are pooled (650 cGy host irradiation), the Tc2 population was found to abrogate the rejection of TCD marrow (+Tc2 > marrow alone;  $P = .01$ ) and found to prevent marrow rejection more potently than Tc1-type cells (+Tc2 > +Tc1;  $P = .008$ ).

As Fig 4 shows, mice irradiated at 600 cGy that received marrow supplemented with Tc2-type cells were uniformly engrafted with the F1 marrow (F1 chimerism in 5 of 5 recipients; 64%, 66%, 71%, 78%, and 84% F1 chimerism); in contrast, 5 of 5 Tc1 recipients (600 cGy irradiation) displayed less than 1% F1 chimerism. Similar chimerism results were also obtained in this experiment at 161 days posttransplant (not shown). This experiment confirms that donor CD8<sup>+</sup> T cells of Tc2 phenotype are enriched for an ability to prevent marrow graft rejection and shows that Tc2 cells are capable of facilitating alloengraftment in the setting of less intensive host preparative regimens.

## DISCUSSION

In this report, we have evaluated the effect of donor CD8<sup>+</sup> T cells of Tc1 and Tc2 phenotype on marrow graft rejection and have determined that the Tc2 subset is particularly potent in its ability to facilitate alloengraftment. The Tc2 subset, which possessed cytolytic function and secreted type II cytokines, prevented the rejection of MHC-disparate marrow in sublethally irradiated hosts and allowed for a significant reduction in intensity of the host preparative regimen. Because we used an F1 into-parent model in these studies, our results demonstrate that Tc2-mediated abrogation of rejection does not require an alloaggressive response against the host. These findings thus confirm that non-host-reactive donor CD8 cells can play an important role in the regulation of marrow rejection and identify the Tc2 subset of CD8 cells as a population particularly enriched in its ability to facilitate alloengraftment.

The marked ability of the Tc2 population to facilitate alloengraftment indicates that the process of graft rejection is quite susceptible to regulation by non-host-reactive donor T cells. Previous marrow rejection studies using unmanipulated donor CD8<sup>+</sup> T cells indicated that non-host-reactive CD8 cells were less potent than donor CD8 cells capable of mediating an alloaggressive response against the host.<sup>14</sup> In this study, we have demonstrated that the non-host-reactive mechanism for preventing graft rejection can be augmented by using in vitro-generated donor CD8 cells enriched for cytotoxic function and type II

cytokine secretion. Because host-reactive T-cell responses result in GVHD, use of Tc2-type donor populations to abrogate rejection via a non-host-reactive pathway represents a new strategy for improving the balance between alloengraftment and GVHD. In previous studies, we have demonstrated that host-reactive CD8<sup>+</sup> T cells of Tc2 phenotype can mediate a GVL effect with reduced GVHD relative to unmanipulated donor T cells<sup>8</sup>; given these results, we would predict that host-reactive Tc2 cells might also represent a strategy for preventing rejection with reduced GVHD.

Previous studies have indicated that cytolytic function and an ability to clonally delete allospecific precursor CTL might be two parameters predictive for an ability to prevent graft rejection by a non-host-reactive, veto-type mechanism.<sup>12</sup> Given that the Tc1- and Tc2-type donor CD8<sup>+</sup> T cells possessed similar cytolytic function and ability to clonally delete allospecific precursor CTL, we reasoned that these CD8 subsets would prevent marrow rejection in this F1 into-parent model with similar efficacy. However, because the Tc2 subset was clearly superior in preventing marrow graft rejection, we believe that other functional characteristics must contribute to the in vivo effectiveness of this population.

The mechanism for the enhanced ability of the Tc2 subset to abrogate rejection relative to the Tc1 population is currently not known, but likely involves a noncytolytic characteristic of the Tc2-type cells. One possibility is that the Tc2 subset has a longer in vivo half-life or a favorable in vivo homing pattern. A second possibility is that the dichotomous cytokine secretion pattern of the Tc1 and Tc2 subsets might exert differential effects on the graft rejection process in vivo; eg, a murine tumor model has demonstrated that the type II cytokine IL-10 can block an allogeneic rejection response.<sup>26</sup> However, we do not favor this explanation in our experiments, because our previous pilot studies using CD4<sup>+</sup> Th2 cells, which secreted the type II cytokines but were noncytolytic, did not abrogate marrow rejection in an F1 into-parent model.

It is also possible that the Tc2 cells might be more effective than the Tc1 population at preventing marrow rejection mediated by host CD4<sup>+</sup> T cells. The graft rejection model used in these studies involves disparities at both MHC class I and class II alloantigens; as such, radioresistant host CD4<sup>+</sup> and CD8<sup>+</sup> T cells may both have contributed to the marrow rejection process. In contrast to human CD8<sup>+</sup> T cells, which can express HLA class II antigens, murine CD8<sup>+</sup> T cells are not known to express class II antigens; the Tc1 and Tc2 populations used in these studies were negative for MHC class II expression by routine FCM analysis. Because the classical veto mechanism is driven by host recognition of donor antigens, if Tc2 cells are capable of preventing CD4-mediated rejection, an alternative (non-veto) mechanism would presumably be operative.

Clinical translation of the non-host-reactive Tc2 strategy would require the development of methodologies for the generation and characterization of human Tc2 cells that are rendered nonreactive to host alloantigens. Application of this strategy in the setting of transplantation for nonmalignant diseases would likely result in a favorable balance between engraftment and GVHD. However, because the GVL effect may require an alloaggressive T-cell reaction, the use of host-

reactive Tc2 cells for the purpose of abrogating rejection might be advantageous in the setting of leukemic hosts.

In conclusion, we have identified the Tc2 subset of donor CD8<sup>+</sup> T cells as a population particularly enriched in its ability to abrogate marrow graft rejection. Our observation that the Tc2 population abrogates rejection without inducing an alloaggressive response helps define the biology of T-cell regulation of marrow graft rejection and offers a new strategy for achieving alloengraftment with reduced GVHD. Combined with our previous findings that host-reactive Tc2 cells can mediate a GVL effect with reduced GVHD, the current results suggest that donor cells of Tc2 phenotype may be the optimal CD8<sup>+</sup> T-cell subset for use in the setting of allogeneic bone marrow transplantation. Marrow supplemented with both host-reactive and non-host-reactive Tc2 cells might optimally mediate antileukemia effects and prevent marrow rejection. As such, both cytokine phenotype (type I v type II) and specificity (host-reactive v non-host-reactive) of donor CD8<sup>+</sup> T cells are important considerations in attempts to broaden the future clinical applicability of allogeneic bone marrow transplantation.

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